# Physiological-genetical studies on seed germination.

by

T . W . BROWN, B.Sc. (Edin).


Thesis presented for the degree of Doctor of Philosophy, University of Edinburgh.

June 1935.

Title.

Physiological-genetical studies on seed germination.

Contents.

## Chapter I.

# I N T R O D U C T I O N.

Earlier work on germination has always taken an extended view of the process of germination. The process was regarded as commencing when the mature dry seed is subjected to suitable conditions and ending when the young seedling appeared. In most of the earlier work the appearance of the seedling was taken in the soil and this of course also included establishment of the plantlet.

It is clear that the greater part of the process discussed in these researches includes growth purely and not germination in a stricter sense. Work in this laboratory has tended to narrow the definition and regard germination as taking place in three overlapping stages with a comparatively smooth progression from the dry seed to the activated embryo. In the order of their appearance the three stages may be said to be (Nelson and Macsween 1933):-

a) Hydration of the colloids of the testa of the seed;

b) Water intake by an osmotic mechanism through the hydrated testa which acts as a semipermeable membrane;

c) "Vital" reactions involving energy release, and movement and reorganisation of plastic materials - more crudely respiration and growth.

It is to be noted that this theory reduces the importance of the micropyle to the vanishing point which is contrary to the generally accepted view given in most text books. It is clear that no stage of the germination process of a matured dry seed can eventuate unless preceeded by the intake of water.

Thus water intake must be regarded as the first step in the process. The end limit must be early in the third stage. The definition of this end point however, is not important in this thesis, since the whole of the work to be reported deals with the earlier stages - water intake. While Nelson and Macsween provided a theory to work upon and a somewhat new conception of the problem, they left a wide field defined but not explored and it fell to the present writer to establish methods of technique and accumulate data bearing on the problem. Thus much of the work here reported is exploratory, and has followed along a number of somewhat diverse avenues.

No matter what line of action was taken during the work the whole bears directly on the problem of what is the mechanism involved in the intake of water by seeds, and how do various material factors affect it.

Material:- As soon as the complexity of the problem was realised a search was made for the simplest suitable material available. The choice fell on the seed of the Broad Bean (Vicia Faba. L.) This seed satisfied certain primary requirements. It was available in quantity commercially, and in the variety "Cropper" a supply of material genetically pure for at least major commercial characteristics was available. Further the individual seed is big enough to be handled as a unit in experimental work, and the structure is

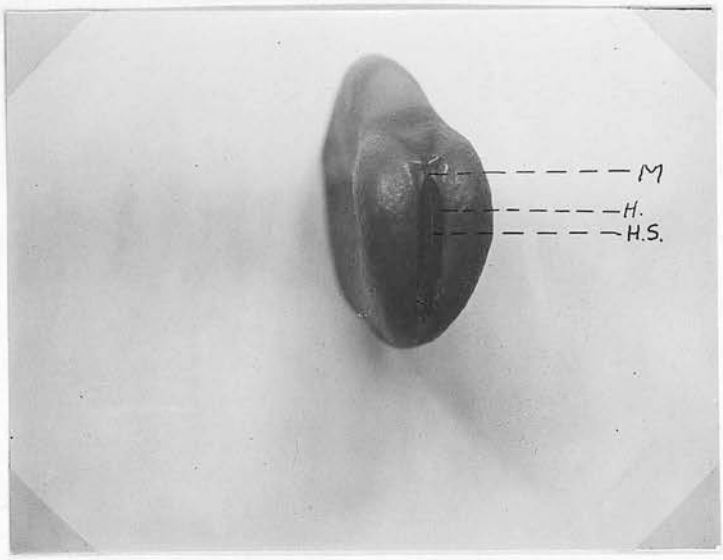


Fig. I
End view of the seed of Vicia Faba. L.
M = Micropyle
H = Hilum
HS = Hilar Slit


Fig. II
Side view of the seed of Vicia Faba. L.
M = Micropyle
H = Hilum
S = Strophile

comparatively simple in that no unorganised substances such as mucillages occur in the tissues. The whole of the results here reported were obtained from experiments with seeds of Broad Bean var. "Cropper" which were purchased from the original raisers Messrs. David Bell Ltd., Leith. To ensure a more perfect understanding of the experiments which are to follow, a brief resumé of the morphology and anatomy of the seed of Vicia Faba. L. may be offered as an introduction to the main thesis.

Figs. I and II illustrate the external appearance of a typical Broad bean seed, the features of importance being the Hilum (H) with the Hilar Slit (H.S.) running along the, centre of the long axis of the Hilum. At one end of the latter is the Micropyle (M), and at some little distance from the other end of the Hilum on the periphery of the seed is situated the Strophiole (S).

On removing the seed coat it is to be noticed that the latter is thickest in the hilar region and that the radicle of the embryo is inserted in a small sleeve like pocket of the testa. The embryo itself consists of two large fleshy cotyledons with the plumule pressed between their two morphological upper surfaces and the radicle pointing obliquely out from the cotyledonary node into the pocket of the testa.

The anatomy of the seed of Vicia Faba L. has been

worked out by a number of investigators. The first worker on this species was Bischoff (1833) who briefly describes the structure of the testa. Pringsheim (1848) published a paper describing in great detail the results of histological investigations on the seed coat of Vicia Faba, and gives some excellent illustrations of the component cells of each layer of the testa. These early workers were later followed by Sempolowski (1874), Nobbe (1876), Beck (1878), Harz (1885), Mattirollo and Buscalioni (1892), and Pammel (1899). The majority of these investigators did not deal specifically with the seed of Vicia Faba, but included it in a comprehensive survey of seed structure. The accounts of seed structure given by them, though they differ in detail, are essentially similar.

The structure of the seed coat over the greater part of the seed is shown in Fig. III. Broadly speaking the testa consists of three main layers. Starting from the exterior these cell layers are:-

1. The Palisade layer.
2. The Hour-glass layer.

and 3. The Nutrient layer.

The cells of the Palisade layer have been variously called Malpighian, epidermal, prism, macrosclerid and palisade cells. Pammel (1899) considered that the name palisade should not be applied, since it is usually used to refer to the elongated thin walled parenchyma cells of the leaf

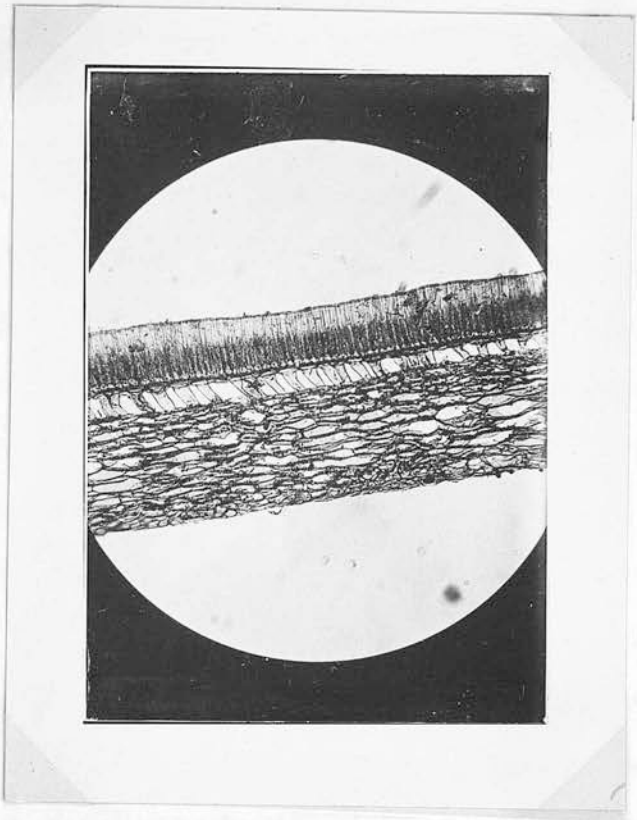

Fig. III
T . S . of the Testa of Vicia Faba. L.

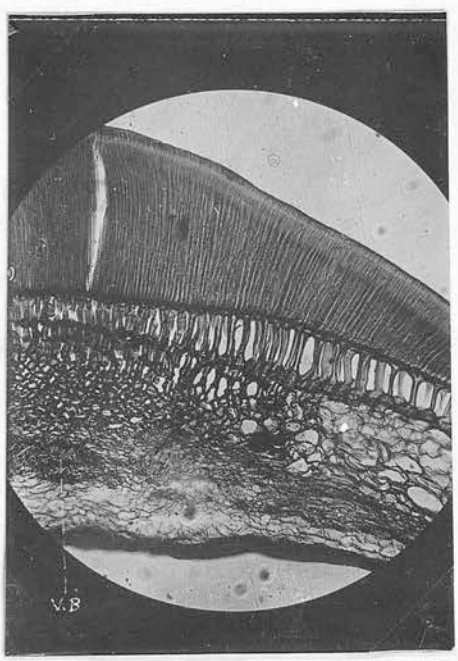


Fig. IV
T.S. of the Testa of Vicia Faba. L. through the Strophiole.
V.B. = Vascular Bundle.

which perform an entirely different function. Pammel therefore favours the name "Malpighian". Throughout the literature, however, the name chiefly given to these cells is "palisade" which was first used by Sempolowski (1874), and his terminology will be adopted here. These Palisade cells are longer than they are broad, with blunted ends, and a cell cavity which is broad at the base tapering to a point at the apex of the cell. The palisade layer of cells is covered by a cuticle, which in section appears as a delicate line of equal thickness. At the end of the Palisade cells towards the exterior there is a highly refractive line extending across the narrow diameter which is called the "light-line." This is undoubtedly the most interesting feature of the Palisade cell. The light-line appears to be a constant character of seed Palisade cells not only in the Leguminosae but also in many other unrelated families. This has given rise to an extensive literature on the subject which can only be briefly referred to here. Its constant appearance in a number of different families renders its significance undoubted, but though many theories have been developed as to its chemical nature, physical properties and functions, nothing definite is yet known regarding it. Sempolowski (1874) considered that there is a chemical modification and a difference in molecular structure in the cell wall at this point. His belief was based on the

observation that with iodine and sulphuric acid the cell wall colours blue, but the light-line becomes yellow. Harz (1885) believed with others that the cell wall at the light-line contained less water and that its appearance is due to physical changes taking place in the laying down of the cell-wall substances. A third theory (Overhage 1887) which has been put forward is that the light-line is lignified, but so far as the present writer is aware no worker using seeds of leguminous plants has supported this hypothesis.

The cell walls of the Palisade cells consist of cellulose, and according to Pammel (1899) the cells contain tannin.

As in the case of the Palisade cells, the hour-glass cells have received a variety of different names. The shape of the cells suggested the name "Sandhurzellen" to Harz, "Säulenzellen" to Sempolowski, and "Collonne" to Mattirollo and Buscalioni. A number of writers have referred to them as "Tragerzellen" meaning "support cells", while Pammel calls them "Osteosclerids." The name "hour-glass" cells is the one most frequently used, and this name will therefore be employed here. The hour-glass layer consists of a single layer of thick walled cells which are narrow in the centre and broaden out at base and apex. There are large prismatic intercellular spaces between the cells. The cell walls which are cellulosic are prominently

marked with longitudinal canals. The cell of this layer according to Pammel contain an abundance of tannin.

The name "Nutrient layer" has been given to the layer inside the hour-glass layer to indicate its function in the immature seed, where the cells of the nutrient layer contain water and food materials. In the mature seed the cells are collapsed and the cell cavities are represented by mere lines. On soaking, however, the cells of the nutrient layer regain their normal shape and the layer as a whole expands considerably. The cells are large and thin walled, though there is a tendency for them to become smaller and thicker walled towards the inner limit of the layer. Bischoff, Sempolowski, and Beck each made statements to the effect that the innermost part of the testa is endosperm. Harz and Pammel considered the endosperm is absent or only very sparingly developed.

Such is the general structure of the testa over the greater part of the seed. It modifies considerably atcertain places. Fig. 1V is a photograph of a section through the strophiole of the seed coat. On the right of the strophiole the structure is not unlike that seen in Fig. 111, but to the left of the photograph the palisade cells are twice their normal length, the hour-glass cells are much reduced in size and the nutrient layer has changed out of all recognition. The anatomy of this

particular portion of the testa seems to have been neglected to a certain extent for the writer can find only casual references to it in the literature. The nutrient layer in this region appears to consist of at least three layers. The cells of the outermost layer abutting on to the hour-glass layer are very small and densely brown in colour which may be accounted for by the presence of large quantities of tannin. The innermost layer is structurally ill-defined and may represent the remnants of the endosperm. Between these two layers is a layer composed of large, clear, thin walled cells through which runs a vascular tract, This vascular bundle starts from the non-micropylar end of the hilum and travels all the way around the periphery of the seed in the nutrient layer of the testa until it reaches the base of the embryo where it bifurcates and the two branches peter out one on either side of the embryo. The vascular bundle runs immediately under the elongated palisade cells of the strophiole since the latter is situated on the periphery of the seed. Though it cannot easily be seen in the photograph, the vascular bundle which is cut in cross section is indicated at V.B.

Fig. V. shows a section cut across the hilum of the seed coat. Examination of the photograph shows that a double row of palisade cells are present in this region. In each row a light line occurs. The outer row is covered by a thick black coating of material the nature of which is unknown. They

shorten toward the edges of the black scar while the inner row shorten towards the "tracheid island". This is a characteristic bundle of tracheids which vary in length and are strongly lignified. The island is oval in outline and connects at the upper end with the Hilar slit ("Nabelspalte" of the German literature). It will be noticed that the hour-glass layer of cells which is found as part of the testa everywhere else (in the testa) is absent from the Hilar region. In its place however there is a thick band of star-shaped parenchyma with very large intercellular spaces. This tissue surrounds the "tracheid island" and abuts directly on to the second palisade layer. The star-shaped cells contain a large quantity of tannin materials. The large thin walled parenchyma cells seen in Fig. V under the star-shaped parenchymaare the continuation of the nutrient layer.

A median longitudinal section through the hilum at the non-micropylar end will show the beginning of the vascular tract which runs around the periphery of the seed. A photograph of such a section is shown in Fig. VI. A portion of the "tracheid island" in longitudinal section is shown to the left of the photograph. The striations characteristic of vascular elements are clearly seen in this portion of the island. Immediately beneath the latter the curious shape of the star-shaped parenchyma is easily distinguished. Note should also be taken of the

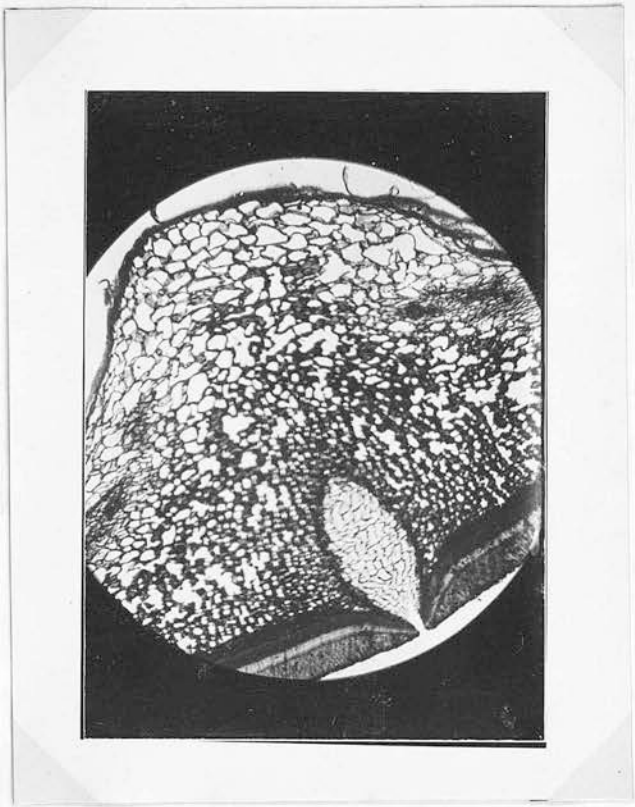

Fig. V.
T.S. across the Hilum of the Testa of Vicia Faba. L.

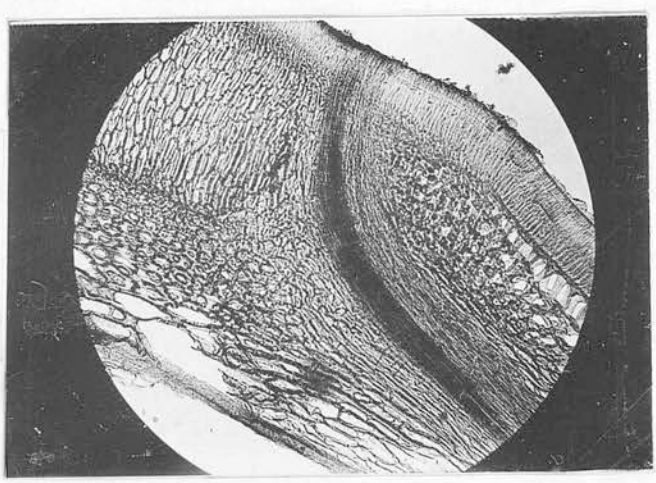

Fig. VI.
Section in the Hilar plane of the non-micropylar end of the Hilum of the Testa of Vicia Faba. L.

continuation of the nutrient layer and also of the fact that the limit of the "tracheid island" is also the limit of the star-shaped parenchyma tissue. To the right of the photograph is seen the reappearance of the hour-glass layer of cells, and with their reappearance the seed coat in this portion of the photograph shows the structure which exists over the greater part of the testa, except that here the vascular bundle is shown running through the nutrient layer. The present writer has been unable to identify sieve tubes and companion cells in the vascular tract, though many other workers maintain that they are present. The xylem consists of spirally thickened tracheids. The bundle is surrounded by a parenchyma sheath.

At the other end of the hilum the structure is slightly different. The appearance of the testa in this region is shown in Fig. VII, which is a photograph of a section of the testa at the micropylar end of the hilum cut in the hilar plane. The figure does not show the "tracheid island" which lies to the left, but it does show the normal testa structure starting at the right, and also the apex of the cavity in which the radicle lies. From this cavity there are indications that a canal runs towards the break in the palisade layer which is shown in the photograph. The cells surrounding this canal are very small and deep brown in colour.

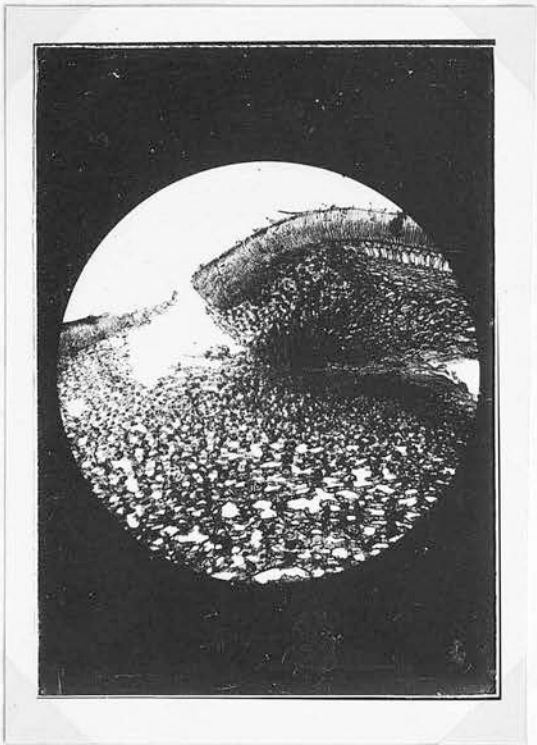

Fig. VII.
Section in the Hilar plane through the Micropyle of the Testa of Vicia Faba. L.

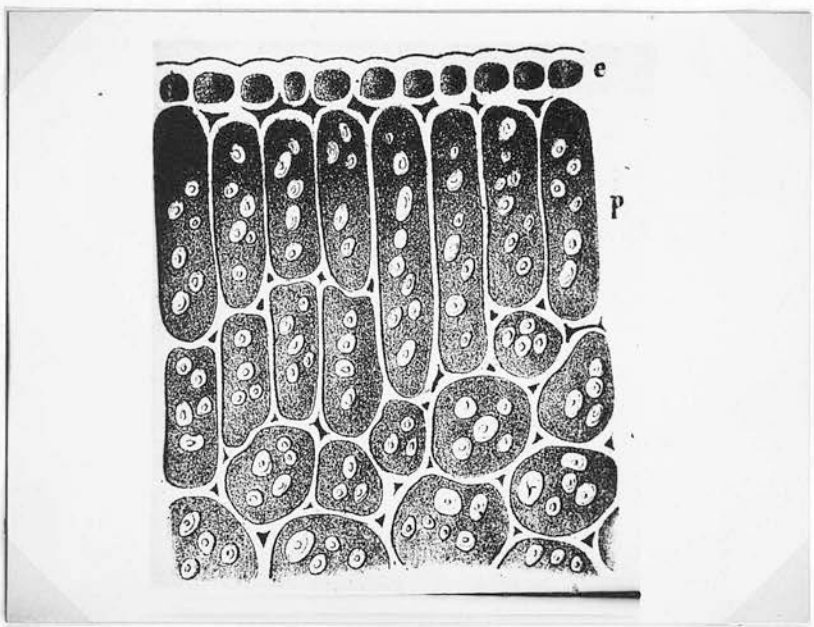


Fig. VIII.
Beck's drawing of a section of the outer surface of the cotyledon of Vicia Faba.L.

There appears to have been some doubt in the past as to whether there was a pore leading from the exterior to the interior of the seed. Sempolowski (1874) working with a number of different seeds states that "Die Micropyle Bildet niemals im reifen Samen ein loch, sondern eine Vertiefung an jener Stelle, wo die Spitze der Radicula liegt; und selbst bei genauer, mikroskopischer Betrachtung gelingt es nicht eine Andeutung des ehemaligen Micropyle - kanals zu bemerken." Beck (1878) working with various species of Vicia and Ervum supports Sempolowski, while Pringsheim (1930) gives drawings of the structure in Lupinus albus showing that a canal does not exist although he does illustrate the break in the palisade layer. Pammel (1899) on the other hand figures a definite passage from the exterior to the interior. Harz (1885) does the same for several leguminous seeds. Recently a worker in this laboratory has shown that there is a micropylor canal in Medicago species. The present writer after examination of a number of sections of this region is convinced that the micropyle which is visible from the exterior as a break in the surface of the coat leads into a small canal which runs into the pocket of the seed coat which houses the radicle of the embryo.

The published accounts of the structure of the testa agree in general with that above given. Differences where they exist have been indicated, but these differences are of minor importance with

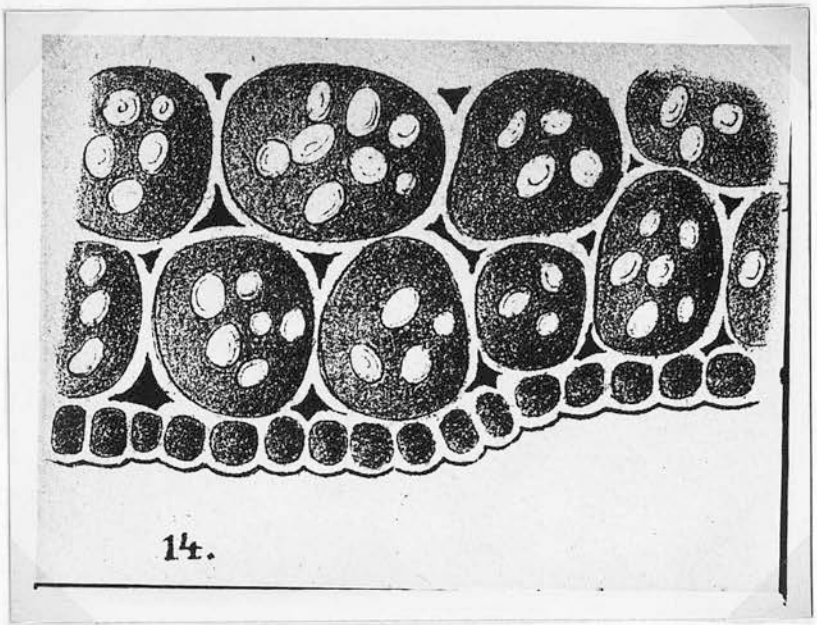


Fig. IX.
Beck's drawing of a section of the inner surface of the cotyledon of Vicia Faba.L.

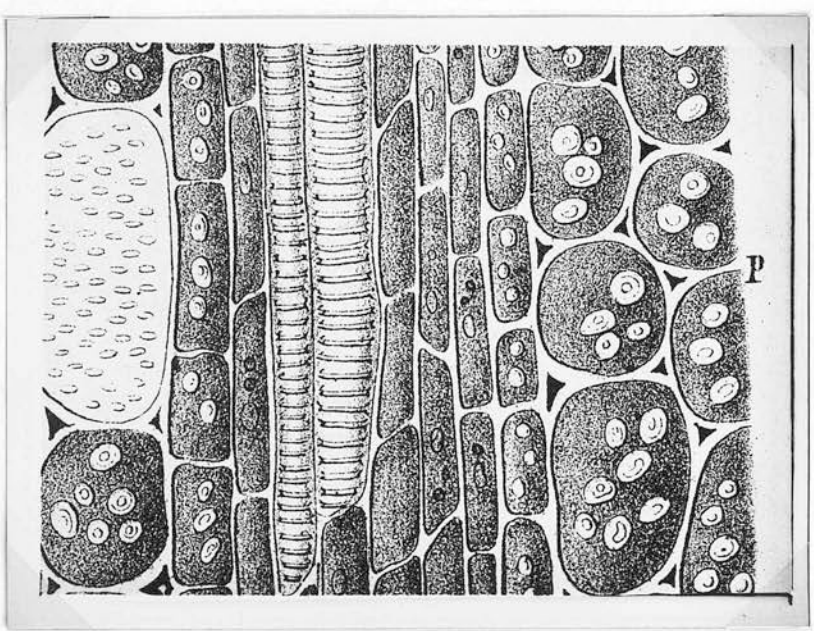


Fig. X.
Beck's drawing of a longitudinal section through a vascular tract of the cotyledon of Vicia Faba. L.

one exception of that in respect of the micropylar canal. The literature on seed coat structure is rich in discussions over small details in anatomy and histology, which however need not concern us since only a broad understanding is aimed at here.

Beck (1878) gives an excellent account of the structure of the cotyledons. His drawings have been photographically reproduced in Figs. VIII, IX and X. The first of these indicates the structure of the outer surface of the cotyledons. It shows clearly the very thick walled small celled epidermis. The underlying layer of cells are elongated and extend vertically into the cotyledon. These prosenchyma cells are followed by thin walled parenchyma cells with intercellular spaces at their angles. Fig. IX shows the internal face of the cotyledon in section. The structure here differs from that of the outer face in that the exterior walls of the epidermis are not so thick, and in this case, there are no elongated cells. Fig. X is a longitudinal section through a vascular tract of the cotyledon. It shows clearly the well developed procambial elements with well formed spiral vessels, surrounded by a bundle sheath. These elements arise as branches from a central bundle which joins on to that in the hypocotyl of the embryo. At the left of Fig. X Beck shows a surface view of a parenchyma cell with porous thickenings. The present writer has been unable to identify these thickenings.

The cells of the cotyledons contain an abundance of starch and rather less protein.

## Chapter II

Physiological and Biochemical aspects of the testa and cotyledons of the seed of Vicia Faba L.

I. Introduction.

II. Methods and Experimental results.

a. Biochemistry of the testa.
b. Biochemistry of the cotyledons.
c. The testa as a membrane.

III. Summary.

## INTRODUCTION

In the course of the general introduction to this thesis it was pointed out that the investigations here reported are based on a hypothesis of water intake by the seed which was formulated as a result of work carried out in this laboratory by Nelson and Macsween (1933). These workers considered that seed imbibition is effected in two steps the first of which is the hydration of the testa colloids, followed by osmotic intake of water through the hydrated testa. The second step presupposes two conditions. (a) that the membrane is a semi-permeable one, and (b) that osmotic substances do exist behind the membrane of the testa. Innumerable papers have been published giving results which show that the testa of seeds is a semipermeable membrane. Results which would indicate that the same is true of the seeds of Vicia Faba are given later on in this chapter. There is however not the same body of evidence in favour of osmotic substances in the seed. Indeed the only two substances which have been reported as existing in the seed and which could act osmotically are Tannins and a reducing sugar. The latter according to Nelson and Macsween (1933) is the osmotically active substance which draws water through the hydrated testa.

The experiments described in this chapter though they are all concerned with the second step in the

hypothesis of Nelson and Macsween, are so different from one another in method that it has been decided to adopt in this chapter an order slightly different from that used for the other chapters. Immediately preceeding the presentation of the results of an experiment the methods used in the experiment will be described, thus dispensingwiththe material and method section of the chapter.

## Methods and Experimental Results.

a) Biochemistry of the testa.

In the course of their investigations Nelson and Macsween (1933) obtained a Fehling reduction test with an extract of the testa of Vicia Faba. Their finding was confirmed by the following experiment.

The seed coats of ten seeds of Vicia Faba were put to soak in about 100 c.c. of distilled water in a tightly corked conical flask. After soaking overnight a sample of the clear brown liquid was decanted and tested with Fehling's solution. A very dense red precipitate was formed indicating that a large amount of some substance capable of reducing alkaline solutions of cupric salts is present in the extract.

Nelson and Macsween made a further statement to the effect that the reducing substance was present in the nutrient layer of the testa. If this is the case it was thought probable that the substance was present in a crystalline or amorphous condition in the dry seed. The testa of an unsoaked seed was stripped off and with a sharp clean scalpel the nutrient layer of the coat was scraped on to a glass slide and a microscopic examination made of the scrapings. After adding a drop of water the scrapings were again examined. In the dry condition there appeared to be no crystals or starch grains and the scraping seemed to consist of nothing more than portions of broken cell walls. With water no

portion of the scraping dissolved. The technique is admittedly crude, but it is sufficiently conclusive to be able to say that the reducing substance, if it does exist in the nutrient layer, is not in the form of crystals though it may possibly exist in an amorphous state that is so fine as not to be noticeable when it is dissolving. Further there is no evidence of starch grains. This point is confirmed by the absence of any gelatinizing grains as the temperature of the water in which the scraping was suspended, was gradually raised to 90°C on a hot stage; the iodine test was also negative.

It was pointed out in the introduction that it has long been known that Tannins are present in the nutrient and palisade layers of the testa. The nature of these tannins however, is unknown. The presence of tannin substances in the testa was confirmed by testing a water extract of the testa with solutions of Ferric salts. Ferric acetate, chloride and sulphate all gave blue-black precipitates. While confirming the existence of tannins in the testa the formation of a blue-black precipitate with Ferric salts indicates that the Tannins present are of the pyrogallol group. The tannins of this group give an orange precipitate with a solution of gelatine, no precipitate with Bromine water, and show no action with concentrated Sulphuric Acid. A water extract of the testa answers to all these

tests and show that the tannins present in the testa are pyrogallic and not catechollic in nature.

Tannins, among many other substances are readily oxidised and are capable of reducing alkaline solutions of cupric salts. In this they have a common property with a number of the monosaccharides, and the question naturally arises, is it a reducing monosaccharide or the Tannin which is responsible for the positive Fehling test obtained with a water extract of the testa?

To answer the question use has been made of the property of monosaccharides to form osazones with phenylhydrazine acetate. A test was carried out according to the method described by Plimmer (1918). The test was carried out for three series each consisting of five test tubes. The contents of the test tubes of each series were as follows:-

(1) Testa extract and distilled water.

(2) Testa extract and 2% Glucose solution.

(3) Distilled water and 2% Glucose solution.

At the end of the test, series (2) and (3) showed large numbers of glucosazone crystals, while the test tubes of series (1) contained no osazone crystals of any kind. It is clear therefore that the reducing substance present in an aqueous extract of the testa is not a monosaccharide.

Nor yet can it be a disaccharide or trisaccharide possessing an aldehydic (-C.OH) group. The reducing

power of the testa extract appears to be due therefore to the presence of pyrogallol tannins.

It is not impossible however that some other disaccharide than one possessing properties of aldoses is present in the nutrient layer, and that this sugar together with the tannins acts osmotically to draw water through the hydrated testa. This however is not the case since elimination of the tannins of a testa extract, with lead acetate followed by hydrolysis with dilute Sulphuric acid and neutralisation with sodium carbonate, gives a solution containing no monosaccharide. In this test the excess lead salt is removed as a precipitate of lead sulphate when the Sulphuric acid is added. A control test containing both tannin extract and sucrose was positive with Fehling's test.

From the experiments above described the conslusion arrived at is that no sugar of any kind exists in the testa of the seed of Vicia Faba, and that the reducing power of the testa extract is due to the presence of pyrogallol tannins.

b) Biochemistry of the Cotyledons.

The presence of sugars in the cotyledons might be responsible for an osmotic intake of water through the hydrated testa, though it could not account for certain osmotic phenomena exhibited by the testa which will be indicated later. In order to ascertain whether sugars do exist in the cotyledons the following experiment was carried out.

Cotyledon meal obtained by putting the cotyledons of a number of seeds through a small hand grinding mill was added to 400 c.c. of distilled water. After boiling one 200 c.c. portion and cooling, the boiled and unboiled portions were each divided into four 50 c.c. quantities. To some of the latter portions in conical flasks, was added a pinch of invertase or a pinch of diastase according to the following arrangement:-

| | | |
|---|---|---|
| (i) | 50 c.c. unboiled. | |
| (ii) | 50 c.c. unboiled | Invertase. |
| (iii) | 50 c.c. boiled. | |
| (iv) | 50 c.c. boiled | Invertase. |
| (v) | 50 c.c. unboiled. | |
| (vi) | 50 c.c. unboiled | Diastase |
| (vii) | 50 c.c. boiled. | |
| (viii) | 50 c.c. boiled | Diastase. |

After incubation for 24 hours at 20°C the contents of each flask were filtered and the filtrate of each tested for reducing sugars with Fehling's solution. (ii) and (iv) gave slight precipitates while (vi) and (viii) gave very dense precipitates.

These results show clearly that sucrose, or some trisaccharide capable of being attacked by invertase, is present in the cotyledons. Further it is concluded that no monosaccharide or reducing disaccharide is present while starch is present in abundance. It should be noticed too that the absence of a reducing substance in (i) and (v) indicates that no emzymes capable of attacking Sucrose or Starch are present in the dry cotyledons.

c) The testa as a membrane

It was pointed out in the introduction to the

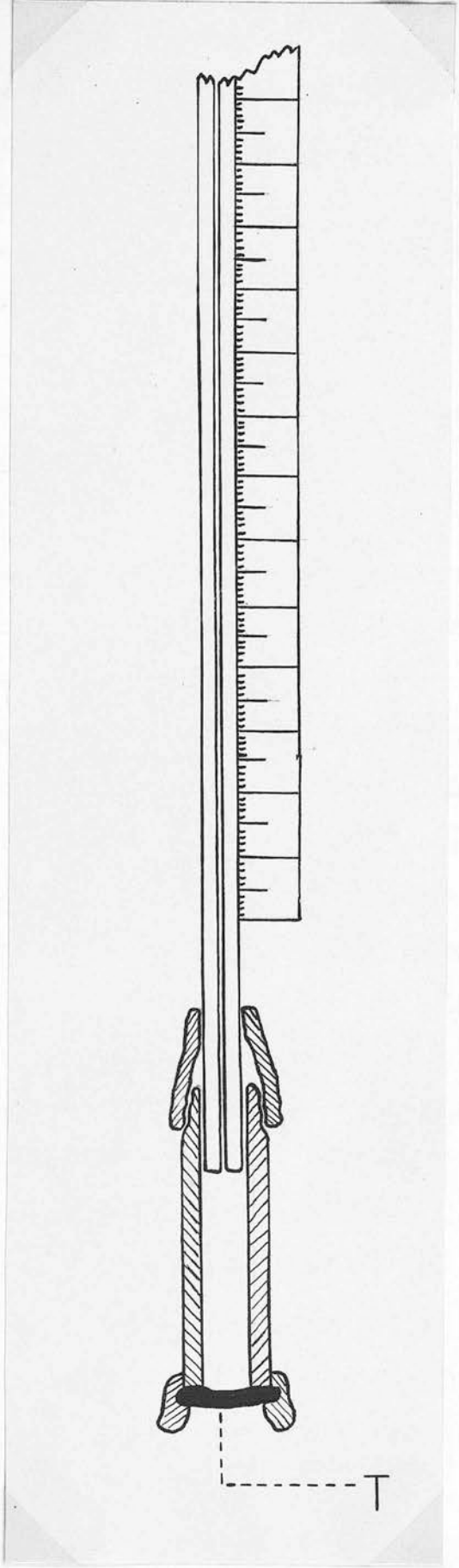


Fig. XI.
Osmometer after Nelson and Macsween (1933).
T = Testa of Seed.

present chapter that a necessary condition of an osmotic intake of water through the hydrated testa is semipermeability of the testa as a membrane. The followingg experiment shows that the testa is in fact semipermeable.

A circular disc of the testa was cut and placed in the osmometer described by Nelson and Macsween (1933). Fig. XI illustrates the main features of this piece of apparatus. After filling the osmometer with a 5% solution of sulphuric acid the lower end of the brass cylinder was lowered in water so that on the outer surface, the testa is bathed with water and on the inner surface with Sulphuric acid. The level of the liquid in the capillary tube, which is provided with a scale and which is attached to the top end of the brass cylinder by means of a piece of rubber tubing, rises gradually to a maximum and thereafter falls. Fig. XII is a graph on which the height of the column of liquid has been plotted against time, and shows clearly the rise and fall indicated above.

Exactly the same thing occurs when water instead of Sulphuric acid is placed inside the osmometer. The changes in the level of the liquid in this case are graphically represented in Fig. XIII. It will be seen that this graph is essentially similar to that of Fig. XII, except that when water was present in the osmometer the maximum reached was not as great as when Sulphuric acid was used.

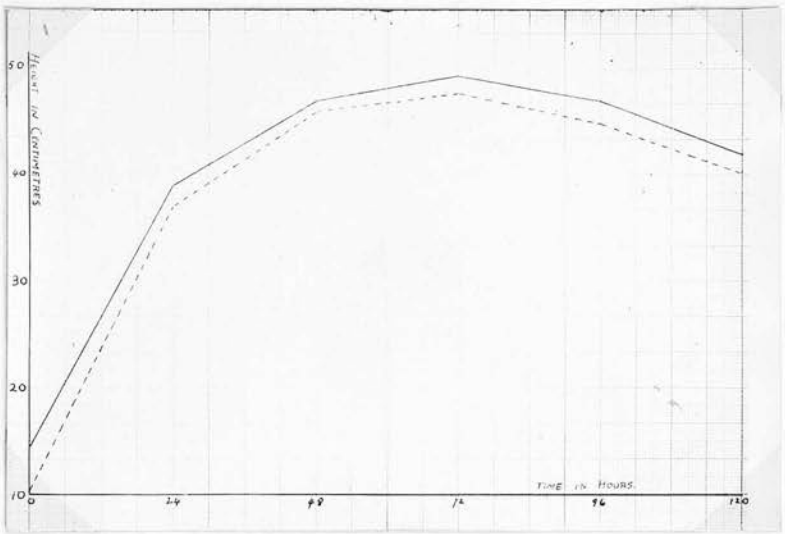


Fig. XII.
Osmometer readings with 5% Sulphuric Acid inside the osmometer.

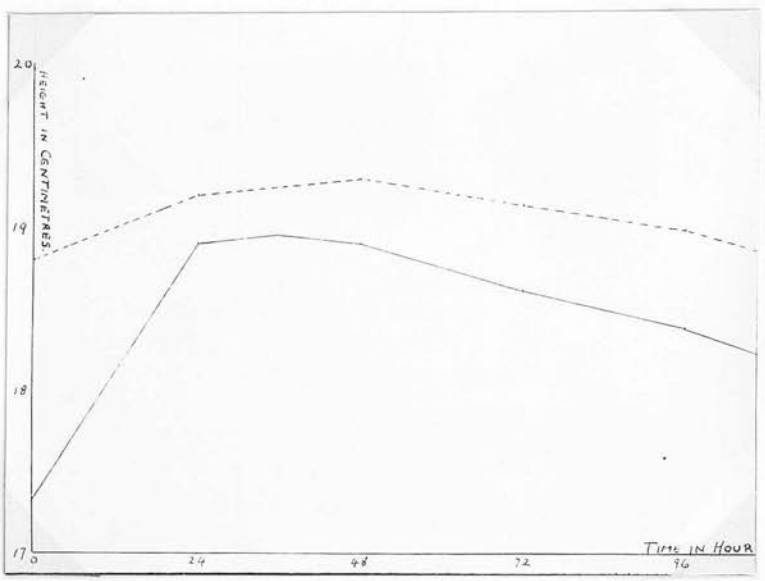


Fig. XIII.
Osmometer readings with water inside the osmometer.

The combined results of these two simple experiments, which have been repeated a number of times always with the same results, enables us to draw some far reaching conclusions. In the first place the rise to a maximum followed by the fall of the liquid in the capillary tube indicates that either the semipermeability of the testa suddenly breaks after a considerable lapse of time during which hydration of the testa colloids has presumably been constantly increasing, or that the testa is at no time a perfectly semipermeable membrane. The writer is inclined to adopt the first view, since the osmotic substances inside the osmometer could only be observed in the external solution after the fall in the level of the liquid in the capillary tube had commenced. This observation has been made with a number of different solutions inside the osmometer. The solutions tested for this purpose included 1% Sucrose, 1% Dextrose, 1% Sulphuric acid, 1% Citric acid and 1% Oxalic acid. In the case of 5% Sulphuric acid however, the water bathing the external surface of the testa disc contained traces of Sulphuric acid some considerable time before the maximum level of the liquid in the capillary tube had been reached. The rise in the level of the liquid in the capillary tube when water is on either side of the membrane is shown later to be due in some measure to the presence of tannin in the testa. When the

column of liquid begins to fall, the tannin begins to diffuse into the external solution. The very small quantity of tannin present in the external solution at this stage was detected by Gold Chloride which gives a brilliant purple colouration in the presence of tannin. The probability is that the sudden break in the semipermeability of the seed coat is due to the enlargement of the intermicellar spaces consequent on hydration of the colloids of the testa. The apparent exception of the 5% Sulphuric acid experiment is explained on this basis by saying that at the time the intermicellar spaces had become large enough to permit Sulphuric acid to pass through to the water bathing the external surface of the membrane, the osmotic pressure of the solution inside the osmometer was greater than the hydrostatic pressure of the column of liquid in the capillary tube and that water diffused into the osmometer until a point was reached when the sum of the hydrostatic pressure of the column of liquid and the osmotic pressure of the external solution was equal to the osmotic pressure of the solution inside the osmometer. This point would be the maximum level reached by the liquid in the capillary tube.

It was at first thought possible that the intake of water through the hydrated testa is not the result of osmotic activity but the result of the exertion of energy pumping water into the seed. The exertion of energy in this way is only possible with a living

membrane, and the fact that a 5% solution of Sulphuric acid does not prevent the intake of water shows that the membrane is dead. The assumption that water intake through the testa is a result of osmotic activity is therefore justified.

When water is placed inside the osmometer a rise in the level of the liquid in the capillary tube takes place. This indicates conclusively that some substance capable of exerting an osmotic pressure is present in the testa behind the semipermeable layer of the testa. The results of the biochemical tests on the seed testa showed that there was no indication of the presence of any sugars in the testa but that there existed an abundance of pyrogallol tannin. This tannin in solution could undoubtedly act osmotically, but no evidence has yet been presented to show whether it alone is responsible for the osmotic intake of water. An experiment was therefore carried out in which the tannins were removed from the field of activity by precipitation with a .25% solution of gelatine placed inside the osmometer. The imbibitional effect of the gelatine was neutralized by dipping the osmometer into a quantity of the same gelatine solution which was protected against concentration through evaporation of moisture by a thin film of paraffin oil. Fig. XIV shows the changes in level of the liquid in the capillary tube for each of two experiments. Examination of these

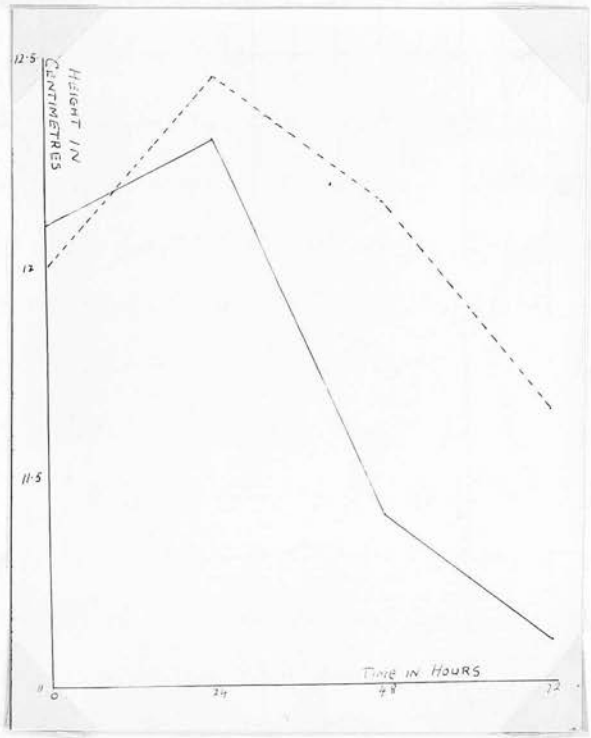


Fig. XIV.
Osmometer readings with .25% gelatine inside the osmometer.

two graphs shows that in spite of the precipitation of the tannins, water has been drawn in through the testa indicating that there is some osmotically active substance other than tannin present in the testa. Nierenstein (1927) gives us a clue as to the possible nature of this substance. In a comprehensive discussion of tannins and their reactions, he emphasizes that while gelatine precipitates all the true tannins it only carries down a small proportion of gallic acid. The latter is a component of most gallotannins, and is often associated with vegetable tannins. It is quite possible therefore that a minute quantity of gallic acid which has not been precipitated by the gelatine remains active in the osmometer and causes the intake of water. Experimental evidences on this point is still lacking owing to the difficulty of evolving a technique which must involve the precipitation of both the tannins and gallic acid without introducing errors.

A final point of interest is made clear by comparing Figs. XIII and XIV. Examination of these two figures shows that even the greatest rise in level in the gelatine experiments is not as great as the smallest rise in level when water is on either side of the testa. In this connection it should be pointed out that a number of experiments were carried out with water on either side of the membrane and that the results of the two experiments

graphically represented in Fig. XIII are those of the experiments which showed the greatest and the smallest rise in the level of the liquid in the capillary tube. The same applies for the two gelatine experiments recorded in Fig. XIV. The fact that when the tannins are removed from solution inside the osmometer the level of the liquid in the capillary tube never reaches the same height as when the tannins are not precipitated, indicates that the tannins are responsible for a considerable amount of the osmotic pressure which draws water through the hydrated testa.

## Summary of Conclusions.

(i) While there is no sugar present in the testa of the seed of *Vicia Faba L.* there is a considerable quantity of pyrogallol tannin.

(ii) Evidence is brought forward to show that the only sugar present in the cotyledon is one capable of being attacked by invertase.

(iii) The testa is a dead semipermeable membrane. Its semipermeability only holds for a limited time. It is suggested that the break in semipermeability is due to the enlargement of the intermicellar spaces consequent on hydration.

(iv) The tannins present in the testa act osmotically to draw water through the testa. The results of experiments discussed show that there is some other substance which acts with the tannins in drawing water through the testa. The suggestion is made that the substance may be gallic acid.

## Chapter III.

The course of water intake of dry seed of Vicia Faba L.

### Contents.

## Introduction.

Our present knowledge of the fundamental mechanism of water intake by seeds is meagre and has been gained largely by techniques which involve following the increase in weight of the seeds during hydration. In this one respect the techniques used by a number of workers is similar, but in other respects the methods adopted vary enormously. There can be no doubt that many of the divergent statements which are found in the literature are due at least in part to differences in the method of arriving at the data. One of the differences in method which has proved most fruitful in the production of contradictory statements is in the length of the time intervals between weighings of hydrating seeds. In some cases the intervals were hourly in others two hourly and in others still, four hourly. The treatment of samples of ten or twenty seeds as units is probably however, the most serious defect in practically all researches on this subject. As will be evident later in this chapter seeds vary considerably from one to the other in their hydration characteristics. Unless it is shown therefore, that the hydration curve of the sample is similar to those of a number of individual seeds, no importance can be attached to the results of researches involving the use of samples.

The theory of the mechanism of water intake put forward by Nelson and Macsween (1933) was based

largely on evidence derived from following the intake of water of dry seeds by weighing individual seeds every four hours after the soaking treatment commenced. The same technique was adopted in the experiments here described, but for reasons which will become apparent later the technique employed here differs in certain essential details from that used by these workers.

Methods and Material.

The individual seeds used in the experiments reported in this chapter were drawn at random from a bulk sample of the "Cropper" variety of Vicia Faba L. Apart from the fact that it was sold as a commercial pure line nothing is known regarding the history of the seed up to the time it was delivered in this laboratory. Since then the seed has been stored in a sack in a cold stone floored room.

For a weight increase experiment a number of seeds were taken from the bulk sample and the dry weight of each taken. Each seed was then placed in a small crystallizing dish containing 15 c.c. of distilled water, and every hour or sometimes every half hour the seed was taken out of the dish and after rapidly but gently removing any adherent liquid with a piece of fine linen it was weighed and put back into its particular dish. In most cases the soaking treatment was continued until no further increase in the weight of the seed took place. Throughout the period of soaking the dishes containing the hydrating seeds were kept at room temperature which varied between 16° and 18°C.

A second series of experiments described towards the end of this chapter concern the changes in volume of a system consisting of water and a single seed. The apparatus used in these experiments is shown in Fig. XV. Essentially it consists of the

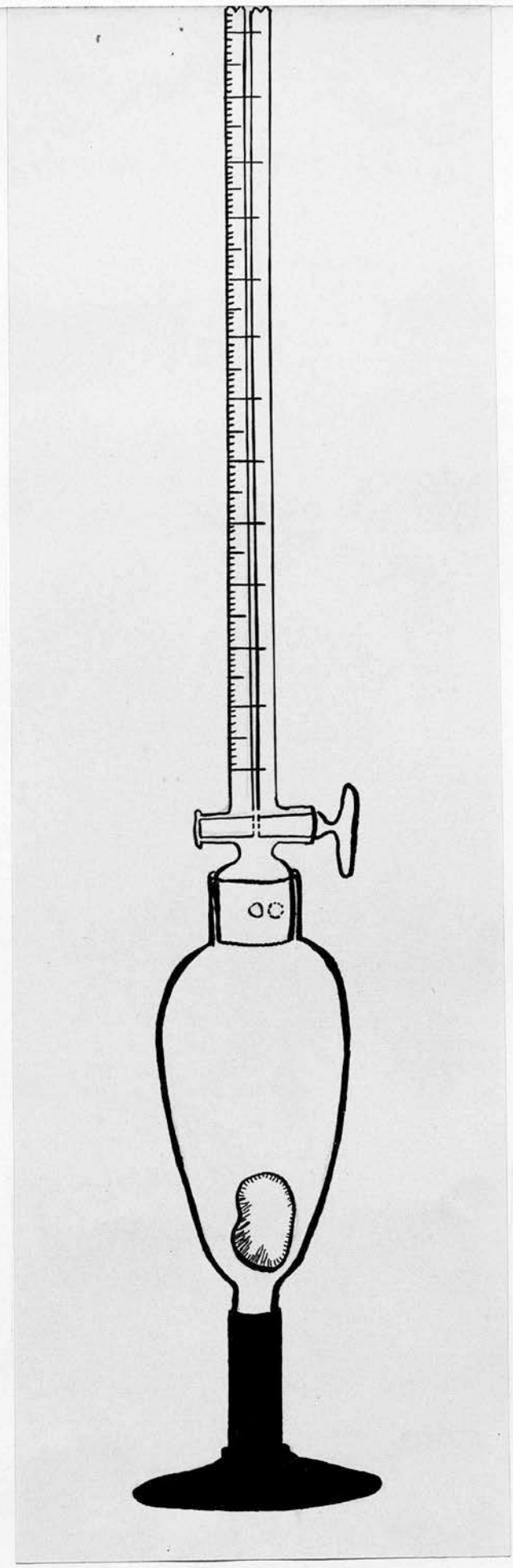

Fig. XV.
Apparatus for measuring changes in volume in a water-seed system.

of the bottom pear-shaped container of a Cannong photosynthometer into the top end of which has been ground a length of graduated capillary tubing. In using this apparatus it was found very necessary to place it in a shielded position where draughts were excluded and where temperature varied as little as possible. The apparatus is sensitive enough to record a change in volume of a quantity of water consequent upon a rise in temperature of $1^{\circ}C$. The apparatus had to be set up under water, and this necessitated having the water in the tank at the same temperature as that of the room in which the apparatus was to stand. This was accomplished by filling an aquarium tank, used for this purpose, with water at least two days before the commencement of an experiment. Readings of the level of the liquid in the capillary tube were taken at intervals.

## Experimental Results.

### a. The increase in weight of seeds during hydration.

For a proper understanding of the sequence of events which take place during the hydration of the seeds, it is necessary first to give a brief account of the changes in external appearance of the seed. On putting a seed into water the appearance of the seed may not change for some considerable time. This interval in time may be absent altogether in some seeds while it may be as long as seven or eight days in other seeds. Eventually, however, the seed coat shows signs of folding (i.e. "wrinkling") at some place on its surface. The particular spot on the seed at which wrinkles first appear varies considerably from one seed to the other. Once wrinkling has commenced it spreads rapidly, the folds increasing in size and number, until the testa in the region of the micropylar end of the hilum has become wrinkled. As soon as this takes place the dewrinkling or smoothening out process of the testa sets in and it progresses very much more rapidly than the rate at which the folds in the testa were formed. The wrinkles having become smoothed out, the seed shows no further change in appearance until the radicle ruptures the coat below the micropyle and protrudes.

The hourly weights of nine seeds from the time of puting into soak to the time of the last increase in weight are given in Table I. These nine seeds

TABLE I. Weight of seeds in grams for each hour of hydration.

| Seed No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Dry Wt. | 1.197 | 1.000 | .914 | 1.070 | .940 | .958 | .700 | 2.180 | 1.033 |
| 1 hour | - | - | - | - | - | - | - | - | - |
| 2 hours | 1.234 | 1.005 | .920 | 1.075 | .941 | .964 | .710 | 2.184 | 1.097 |
| 3 | 1.283 | 1.010 | .917 | 1.075 | - | .966 | .725 | 2.185 | 1.153 |
| 4 | 1.342 | 1.026 | .923 | 1.075 | .943 | .972 | .765 | 2.187 | 1.210 |
| 5 | 1.395 | 1.052 | .938 | 1.076 | .944 | .986 | .826 | 2.189 | 1.260 |
| 6 | 1.443 | 1.101 | .967 | 1.077 | .945 | .998 | .872 | 2.192 | 1.511" |
| 7 | 1.499 | 1.171 | 1.290" | 1.079 | .952 | 1.047 | 1.267" | 2.940" | 1.740 |
| 8 | 1.554 | 1.841" | 2.041 | 1.081 | .962 | 1.081 | 1.490 | 3.609 | 1.926 |
| 9 | 1.606 | 2.803 | 2.493 | 1.090 | .982 | 1.137 | 1.611 | 3.922 | 2.048 |
| 10 | 2.331" | 2.145 | 2.640 | 1.114 | 1.003 | 1.586" | 1.688 | 4.054 | 2.138 |
| 11 | 2.523 | 2.183 | 2.739 | 1.716" | 1.030 | 1.758 | 1.740 | 4.113 | 2.200 |
| 12 | 2.583 | 2.193 | 2.827 | 2.025 | 1.386" | 1.874 | 1.767 | 4.126 | 2.272 |
| 13 | 2.644 | 2.203 | 2.893 | 2.152 | 1.501 | 1.970 | 1.798 | 4.160 | 2.320 |
| 14 | 2.672 | 2.223 | 2.946 | 2.239 | 1.661 | 2.056 | 1.814 | 4.167 | 2.370 |
| 15 | 2.692 | 2.225 | 2.997 | 2.288 | 1.875 | 2.121 | 1.836 | 4.176 | 2.397 |
| 16 | 2.714 | 2.226 | 3.032 | 2.317 | 1.967 | 2.188 | 1.845 | | 2.421 |
| 17 | 2.723 | | 3.057 | 2.340 | 2.008 | 2.279 | 1.864 | | 2.438 |
| 18 | 2.732 | | 3.070 | 2.353 | 2.040 | 2.404 | 1.871 | | 2.451 |
| 19 | | | 3.071 | 2.355 | 2.060 | 2.435 | | | 2.460 |
| 20 | | | | | 2.082 | 2.455 | | | 2.463 |
| 21 | | | | | 2.096 | 2.469 | | | |
| 22 | | | | | 2.106 | 2.479 | | | |
| 23 | | | | | 2.119 | 2.490 | | | |
| 24 | | | | | 2.123 | 2.490 | | | |

" = Micropyle open

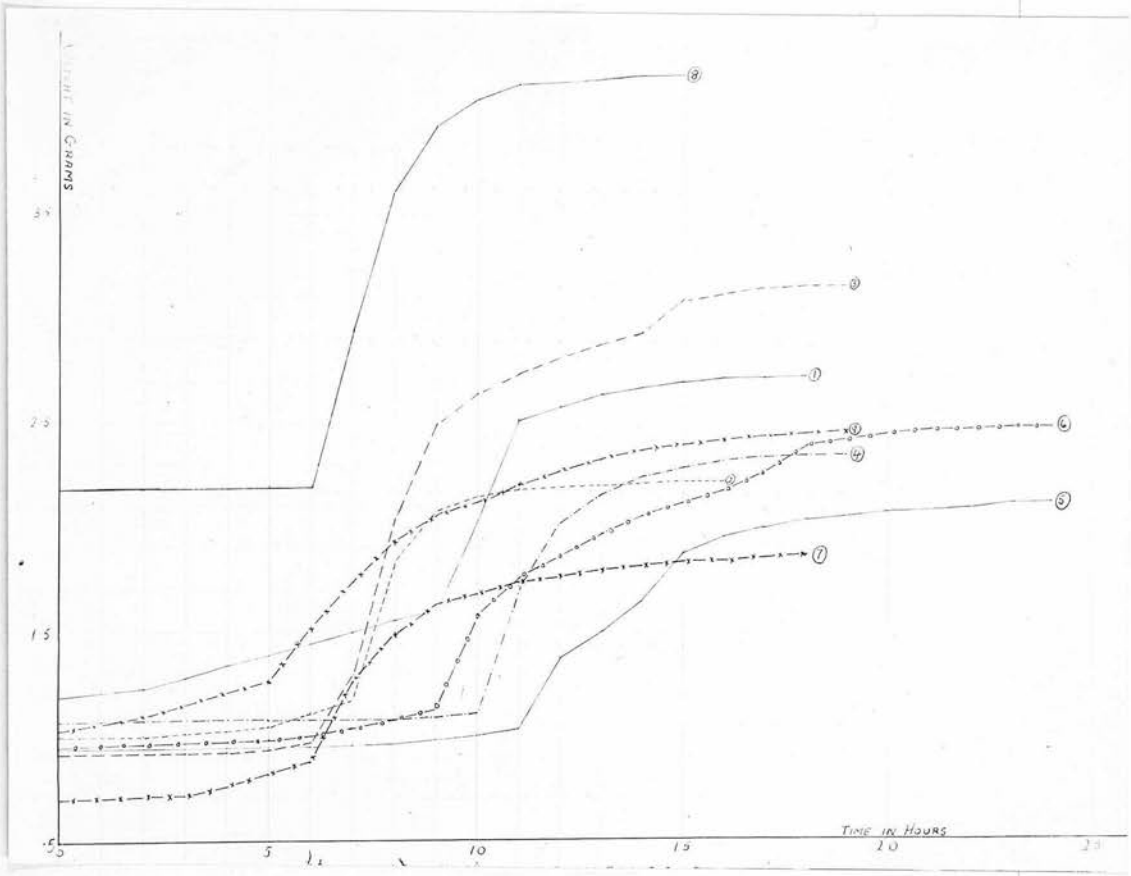


Fig. XVI.
The course of weight increase of individual seeds of Vicia Faba L. during hydration.

represent a selection of a large number of seeds similarly treated. In many of these cases however, the weights are not known for every hour hydration, and have therefore been discarded. Graphs showing the changes in weight for each of the nine seeds in Table I are given in Fig. XVI.

On the basis of weight increase, one feature is exhibited by all seeds during their hydration, and this common feature is clearly seen in the graphs of Fig. XVI. It will be noted that at first the increase in weight of a seed in water is very gradual. It should here be mentioned that the first increase in weight coincides with the first appearance of wrinkles from which it follows that wrinkling is evidence of hydration of the seed coat. The initial gradual increase in weight is followed somewhat suddenly by a phase of very rapid increase in weight of the seed as a whole. It is significant also that the commencement of the dewrinkling process is concomitant with the onset of the second phase of rapid increase. The latter gradually merges into a phase of progressively smaller increases until the seed obtains a constant weight i.e. until the seed becomes completely hydrated. The changes in weight above indicated are even more strikingly shown by considering the amount of weight increase for each hour of hydration. These figures for the nine seeds given in Table I are tabulated in Table II and are graphically represented in Figs. XVII a

TABLE II. Increase in weight in milligrams for each hour of hydration of seeds in Table I.

| Seed No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 3rd hour | 49 | 5 | 3 | 0 | 0 | 2 | 15 | 1 | 56 |
| 4th | 59 | 16 | 6 | 0 | 0 | 6 | 40 | 2 | 57 |
| 5th | 53 | 26 | 15 | 1 | 1 | 14 | 61 | 2 | 51 |
| 6th | 48 | 49 | 29 | 1 | 1 | 12 | 46 | 3 | 250" |
| 7th | 56 | 70 | 323" | 2 | 7 | 49 | 295" | 758" | 229 |
| 8th | 55 | 670" | 751 | 2 | 10 | 34 | 223 | 669 | 186 |
| 9th | 52 | 242 | 452 | 9 | 20 | 56 | 121 | 313 | 122 |
| 10th | 725" | 62 | 147 | 24 | 21 | 449" | 77 | 132 | .90 |
| 11th | 192 | 38 | 99 | 602" | 27 | 172 | 52 | 59 | 62 |
| 12th | 60 | 10 | 88 | 309 | 356" | 116 | 27 | 13 | 72 |
| 13th | 61 | 10 | 66 | 127 | 115 | 96 | 31 | 34 | 48 |
| 14th | 28 | 20 | 53 | 87 | 160 | 86 | 16 | 7 | 50 |
| 15th | 20 | 2 | 51 | 49 | 214 | 65 | 22 | 9 | 27 |
| 16th | 22 | 1 | 35 | 29 | 92 | 67 | 9 | | 24 |
| 17th | 9 | | 25 | 23 | 41 | 91 | 9 | | 17 |
| 18th | 9 | | 13 | 13 | 32 | 125 | 7 | | 13 |
| 19th | | | 1 | 2 | 20 | 31 | | | 9 |
| 20th | | | | | 22 | 20 | | | 3 |
| 21st | | | | | 14 | 14 | | | |
| 22nd | | | | | 10 | 10 | | | |
| 23rd | | | | | 13 | 11 | | | |
| 24th | | | | | 4 | 0 | | | |

" = Micropyle open.

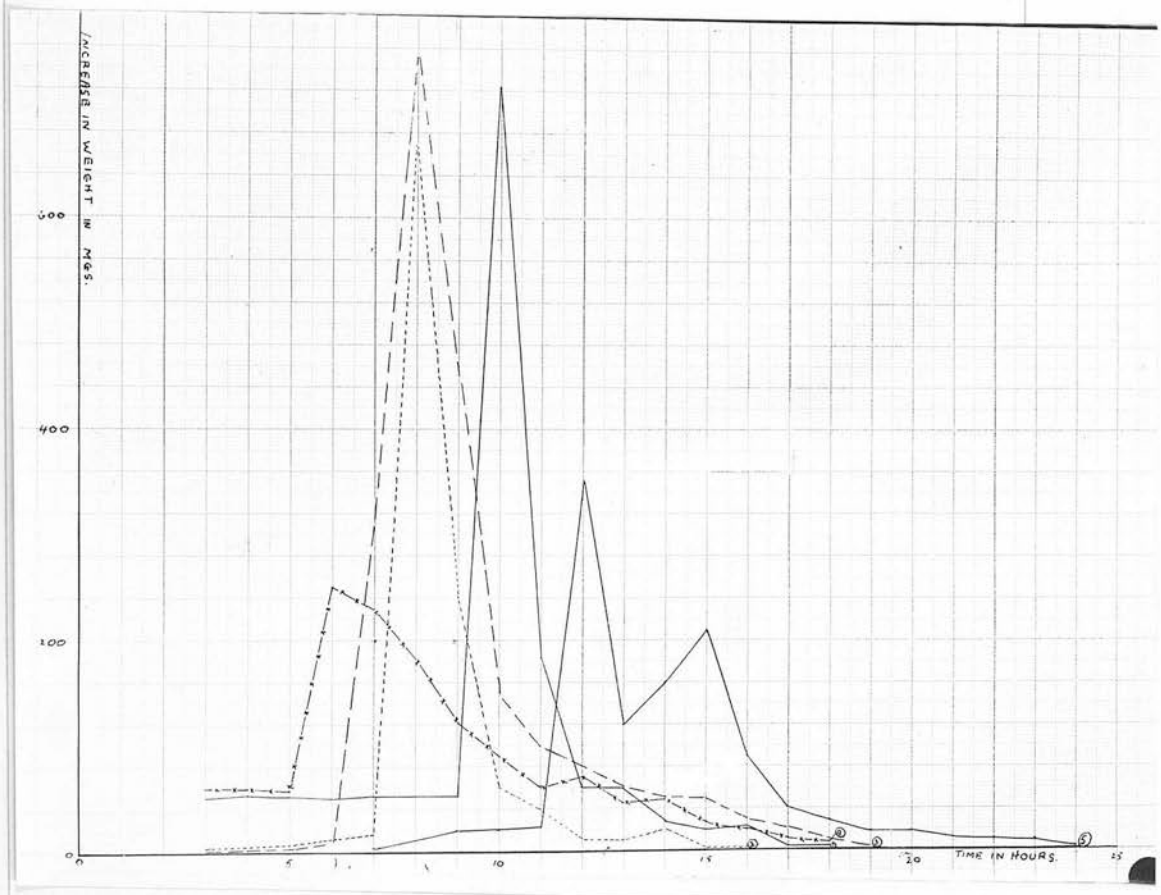


Fig. XVII. A.
Hourly weight increases of individual seeds of Vicia Faba L. during hydration.

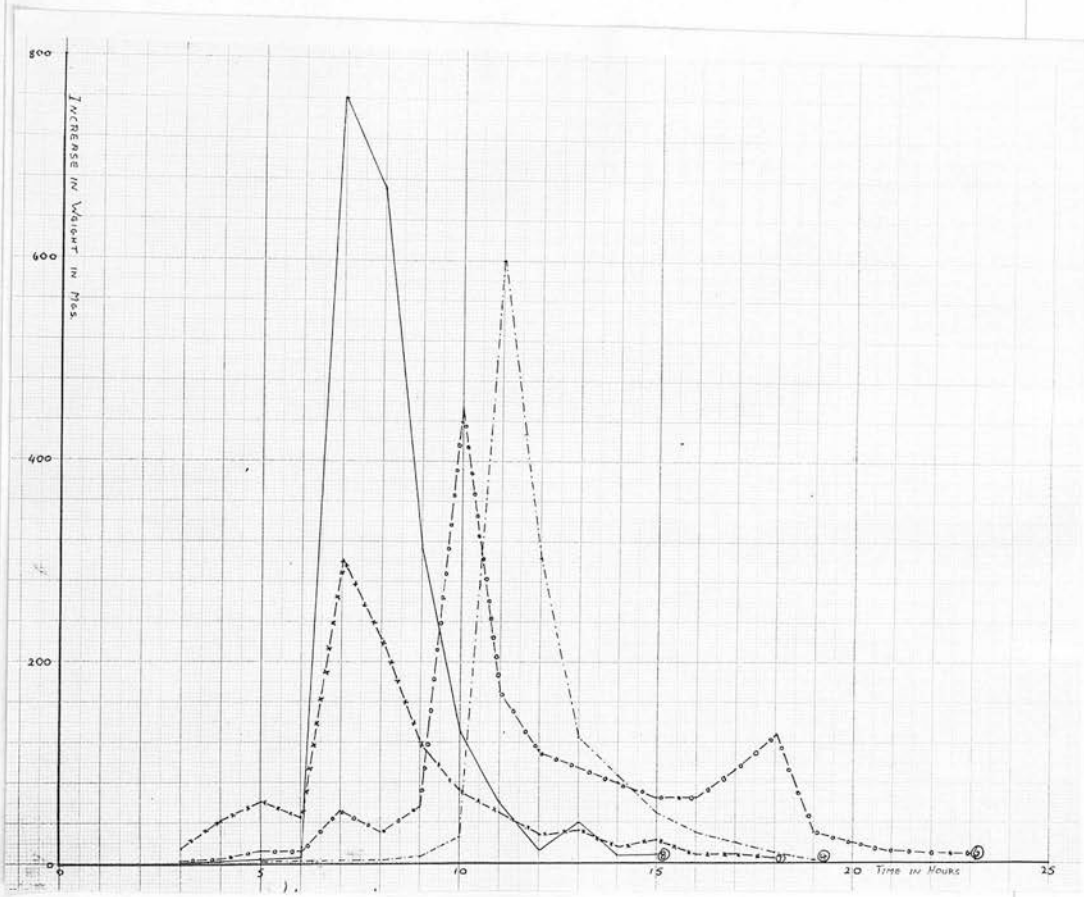


Fig. XVII. B.
Hourly weight increases of individual seeds
of Vicia Faba L. during hydration.

and XVIIb.

The constant appearance of the second phase of rapid increase in weight renders its significance undoubted and makes its explanation all the more important. A clue to the matter was readily obtained in the observation that slight pressure on either side of the seed first causes water to ooze out of the micropyle when the first large increase in weight of the second phase was recorded. Throughout the first phase of gradual increase, the same treatment produces no effect, but with the onset of the second phase the micropyle "opens". The ability to exude liquid is taken as evidence that the pore is open. The hour when the micropyle was first noticed as being open was recorded and is shown in Tables I and II. Examination of these Tables with this point in view will show quite clearly that the time of the first large increase in weight following the initial phase of small hourly increases is exactly the time when the micropyle first opens.

There are two possible interpretations to these results. The first is that the rapid intake of water is responsible for the micropyle being open, and the second is that the opening of the micropyle is responsible for the rapid intake of water. The writer is inclined to support the second possibility. There is undoubtedly some significance in the observation that a prerequisite of the commencement of the second phase of rapid increase is hydration

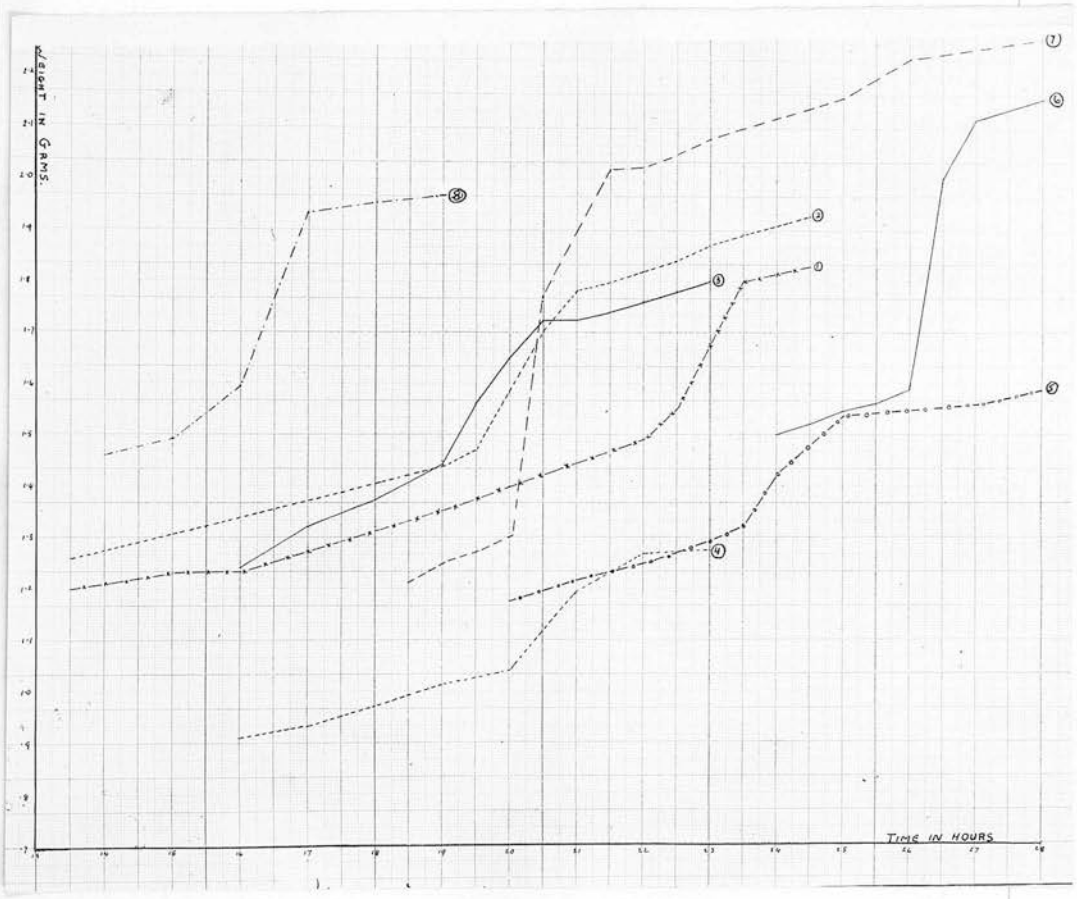


Fig. XVIII.

The course of weight increaseof individual seeds of Vicia Faba L. during hydration, showing the effect of waxing the micropyle.

of the testa tissue in the vicinity of the micropylar end of the hilum. In a later chapter where the individual variation of seeds in respect of time of micropyle opening is discussed this relationship between time of micropyle opening and hydration of the testa tissue in the vicinity of the micropyle is shown to be statistically significant. This in itself is good evidence to justify the supposition that the micropyle has first to "open" before the rapid intake of water characteristic of the second phase in hydration takes place. Further there is no doubt that water does pass through the micropyle. This was shown by the blocking up the micropyle with a plug of wax an hour after the micropyle opened. Waxing of the micropyle at this time had the effect of markedly arresting the rate of increase in weight of a seed. The graphs of Fig. XVIII, which are drawn from the figures given in Table III, show the arrestment in the increase in weight as a result of the waxing of the micropyle during the second phase.

While experimentation on this problem was still in its early stages, and while an interpretation for the phenomenon above indicated was still being sought a suggestion was put forward to the effect that the cotyledons might in some way be connected with the matter. Accordingly an experiment was carried out in which paired cotyledons, that had been carefully removed from the enveloping testa so that the surface of the cotyledons was not damaged, were

TABLE IV. Weight of paired cotyledons in grams for each half hour of hydration.

| Cotyled No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Dry Wt. | 1.100 | .966 | 1.040 | 1.288 | 1.514 |
| ½ hour. | 1.238 | 1.146 | 1.265 | 1.506 | 1.725 |
| 1 " | 1.343 | 1.270 | 1.387 | 1.637 | 1.842 |
| 1½ " | 1.440 | 1.380 | 1.526 | 1.760 | 1.994 |
| 2 " | 1.570 | 1.526 | 1.706 | 1.906 | 2.166 |
| 2½ " | 1.722 | 1.652 | 1.870 | 2.060 | 2.370 |
| 3 " | 1.855 | 1.758 | 2.023 | 2.185 | 2.585 |
| 3½ " | 2.004 | 1.864 | 2.150 | 2.360 | 2.738 |
| 4 " | 2.090 | 1.952 | 2.209 | 2.437 | 2.902 |
| 4½ " | 2.204 | 2.022 | 2.262 | 2.501 | 2.979 |
| 5 " | 2.198 | 2.023 | 2.300 | 2.540 | 3.026 |
| 5½ " | 2.200 | 2.031 | 2.310 | 2.550 | 3.070 |

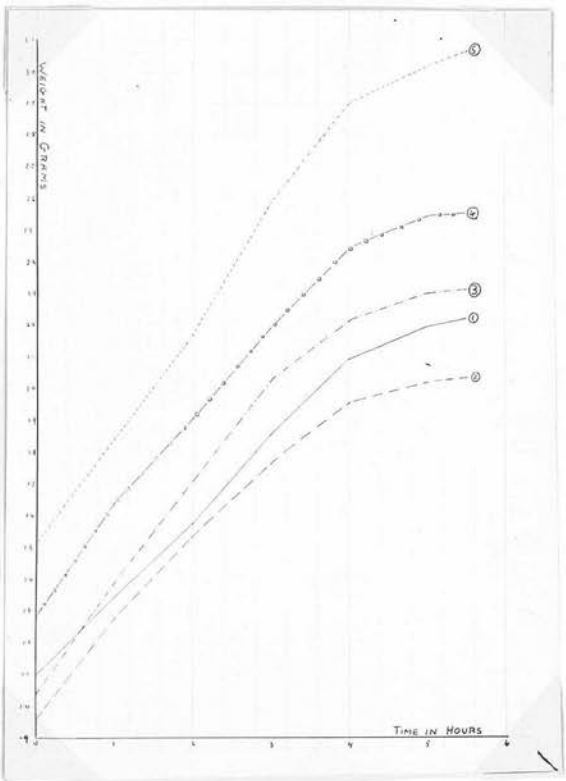


Fig. XIX.
The course of weight increase of paired cotyledons during hydration.

put into the same soaking conditions as applied to the whole seed and weighed every half hour. The results of this experiment are given in Table IV. The figures are graphically represented in Fig. XIX. It will be seen that the cotyledons by themselves show no resemblance to the seed as a whole in their hydration curves. The former increase rapidly in weight from the start, gradually decreasing as hydration proceeds until a constant weight is reached. The curve closely resembles that given by a physically homogenous colloid such as gelatine.

While it has been established that water passes through the micropyle, no mention has been made of the force which draws water through a pore which at most cannot be more than capillary in size. Although there is no direct evidence available on this point there is one possibility which suggests itself in this connection. It is well known that when a colloid hydrates it expands considerably. When the testa hydrates it expands in surface area and would naturally lift off the surface of the cotyledon were it not for the fact that a partial vacuum would then form between the testa and the cotyledon since there is nothing to occupy the space except the gas which was originally present in the seed. The testa therefore goes into folds. The formation of wrinkles necessitates the expansion of the upper half and the compression of the lower half of the palisade cells at the peak of a fold and the

compression of the upper half and expansion of the lower half in the troughs. It has long been known that cells possess considerable elasticity, and the additive force of a number of cells compressed in one portion and expanded in another must be quite considerable. It is here suggested that this force is great enough to draw water through the micropyle when the latter opens as a result of hydration of the tissues surrounding it. This hypothesis has been criticised by workers in this laboratory on the grounds that liquid is present between testa and cotyledons when the former is wrinkled. This is at once admitted if the seed is examined after the micropyle has opened, but prior to micropyle opening the writer has been unable to find free liquid between the testa and the cotyledons. Further it has been shown that once in contact with water the cotyledons hydrate rapidly and this hydration is naturally accompanied by expansion. If free liquid is found between testa and cotyledons prior to micropyle opening why is it that the latter do not hydrate and fill up the space made as a result of the expansion of the testa? The contention here made that no water is present between testa and cotyledons is supported by the results of the changes in volume of the seed-water system a description of which will be given shortly. On the whole the evidence so far available fits in with the hypothesis above developed to account for the force drawing water into the seed

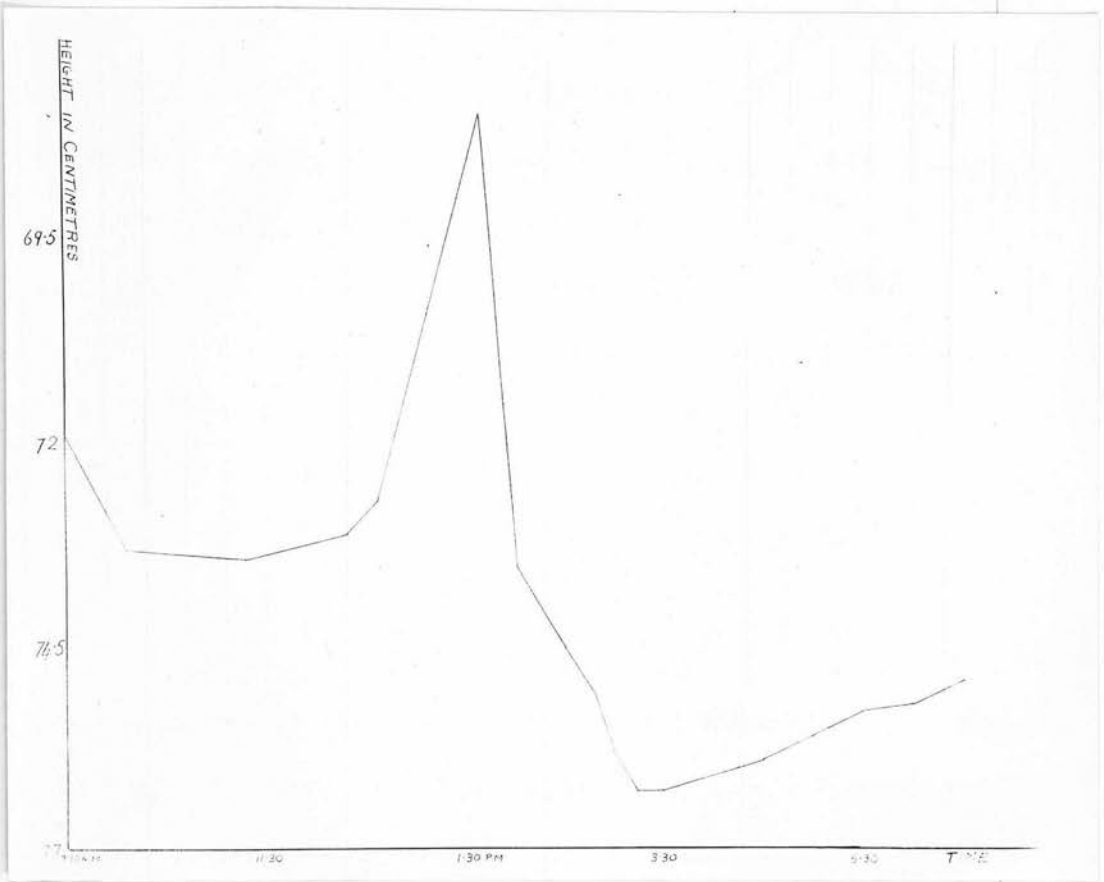


Fig. XX.
Changes in volume of a water-seed system during hydration of a seed of Vicia Faba L.

The latter could not be replaced and thus replication was rendered impossible. The results of these two experiments, however, though requiring confirmation, are sufficiently interesting for discussion here.

The changes in the seed-water system during the hydration of a seed are shown graphically in Fig. XX. It will be seen that after a preliminary contraction the volume of the system increased rapidly and then just as suddenly decreased to a minimum which was followed by a gradual rise in volume. During the course of the experiment the stages in hydration as indicated by the external appearance of the seed were noted. During the initial stage of contraction in volume the seed showed no visible change, but with the beginning of the increase in volume the seed coat showed signs of wrinkles on the periphery of the seed. These wrinkles increased in number and size as the volume of the system became greater and greater. As the volume decreased so the folds in the seed coat straightened out and disappeared until the portions of the testa which had been wrinkled were completely smooth. The rise in volume from the minimum was not accompanied by any externally visible change in the seed.

These results are interesting in that they corroborate the evidence already presented in support of the hypothesis earlier formulated of the mechanism of water intake by the seed. In the weight experiments it was shown that the first increase in weight

of the seed was accompanied by the formation of wrinkles on the seed coat. The volume experiments show that at this same stage there is an increase in volume of the seeds which is out of all proportion to the amount of water being absorbed by the seed. In other words hydration results in an increase in volume at the wrinkled stage, but the increase in volume cannot be accounted for by the amount of water present in the seed. The dewrinkling process it was shown follows the opening of the micropyle and the consequent inrush of water. Here the dewrinkling process is accompanied by a sudden contraction in volume of the system due to the sudden intake of water by the seed and therefore to the contraction of the volume of water surrounding the seed. This agreement is important in that it constitutes clear proof that the squeezing technique used to determine the time of micropyle opening had no effect on the subsequent hydration of the seed.

In the last section the statement was made that during wrinkling prior to micropyle opening there was no liquid between the testa and cotyledons. The statement is supported by the results of the above experiments. The expansion in volume of the seed during the wrinkling period is not directly due to the presence of water in the seed. If water were present in the folds of the testa the volume of the water - seed system would have remained more constant than it actually does during the wrinkling process.

## Summary.

I. It is shown that the first increase in weight of the seed is accompanied by the formation of wrinkles on the surface of the seed and an increase in volume of the seed which is out of all proportion to the amount of water absorbed.

II As soon as wrinkling has reached the micropylar end of the hilum, the micropyle opens and water rushes in through the micropylar canal.

III With this much increased rate of intake of water the wrinkles disappear, and thereafter there is a progressive decrease in the rate of intake until the seed becomes completely hydrated.

IV It is shown that the cotyledons resemble a homogenous colloid in their absorption curves.

## Chapter IV.

## The Individuality of the seed.

### Contents.

## Introduction

As has been noted earlier there are very definite indications that the individuality of the seeds of Vicia Faba is very great. The fact has also been noted by other workers in this laboratory. It was thought desirable and necessary to elucidate the problem of individuality and an endeavour was therefore made to track down as far as possible the causes of the hydration idiosyncracies of individual seeds. Some seeds reach their point of maximum hydration rapidly and others slowly; some seeds show a high initial intake rate and others a low initial intake rate; some seeds at full hydration absorb more water per unit of air dry weight than others; some seeds begin to wrinkle at one point of the testa and other seeds at other loci, and so on. Such differences, it was felt, would have to be accounted for before any reliance could be laid on results of subsequent work. It was also thought possible that some of these hydration characteristics, of seeds would be reflected in the subsequent development of the plant, and to this end the seeds were germinated and grown on.

## Material and Methods.

The seeds used for this investigation were all harvested from plants grown at the Royal Botanic Garden, Edinburgh. Each plant was given a number, and each pod on that plant was given the number of the node to which it was attached, the first flowering

node working from the base of the plant to the apex being number 1. Within the pod, the seeds were numbered starting from the stalk end that is they were numbered from proximal to distal end of the pod. In this way every seed experimented with had a number which at once tells us the parent plant, the position of the seed within its pod, and from which node on the parent the pod came from. For example a seed with the number 40. 2. 4: the first figure 40 signifies the number of the parent plant, the second figure 2 indicates the number of the pod on that plant, and third figure 4 indicates that we are dealing with the fourth seed in the pod.

The parent plants from which the experimental seeds were obtained were all sown on the 18th May 1934. On the 11th of October 1934 the plants were pulled out of the soil and brought into the laboratory. They were then separated into three groups of equal numbers of plants and treated as follows: the seeds of the first group were taken out of the pod carefully and each placed in a small seed packet perforated with small holes upon which was noted the number (i.e. Plant, pod, and position in the pod) of the seed it contained. From the second group of plants the pods were removed and around each pod a label was tied, and on the label was noted the number of the parent plant and the number of the node from which the pod arose. The mud was washed off the roots of the plants of the third group and were otherwise left

untouched, except for the tying of a label round the stem of each plant. On each label was noted the number of the plant to which it was tied. The seeds in the packets, the labelled pods and the labelled plants were then left exposed to the laboratory atmosphere.

The laboratory atmosphere is dry with a range of temperature between 15-20°C.

On the 8th of December 1934, approximately 20 seeds were shelled from each of the second and third groups, and 20 seeds of the first group were taken out of their seed packets. Each seed has a number as previously described, and further observations for each seed were noted against its number. The seeds were individually weighed and each placed in a small crystallizing dish containing approximately 15 c.c. of distilled water. Every hour thereafter for 24 hours each seed was taken out of its dish, the surplus moisture adhering to the coat very gently wiped off with a piece of well washed linen, weighed and placed back into its dish of water. After 24 hours this was done at less frequent intervals. As soon as a seed had ceased to absorb more than .005 grams of water per hour it was taken as having fully hydrated. It was taken out of the water, sown in sand, and labelled with its number. Every day each seed was taken out of the sand and examined to see whether it had germinated. A seed had germinated when the tip of the radicle could be seen. The

seeds that had germinated were each planted in a separate pot. The depth to which seeds were planted below the surface of the soil was kept approximately constant by means of a stick with a collar. The date of the appearance of the plumule at the soil surface was noted and the total height 30 days after germination was noted for each plant.

During hydration the portion of the testa which wrinkled first, and the hour at which the micropyle opened was noted for each seed.

A moisture estimation test was also carried out for each of the three groups of seeds. Three seeds were selected at random from each of the groups and each placed in a previously weighed specimen tube and cork. After heating the tubes containing the seeds in an electric oven for 8 days at 105°C, the tubes were placed in a dessicator until they had reached room temperature. The tubes were then corked and reweighed. The loss in weight was taken to represent the moisture content of the air dry seeds.

The methods most frequently employed in the statistical analysis of the results are those outlined by Banister (1929).

Experimental results.

a) The effect on hydration of the method of storage.

Examination of the figures in Tables Va, Vb, and Vc, reveals the fact that the dry weight of the shelled seeds are lower than they are for the other

TABLE Va. Hydration figures for "shelled seeds"

| Seed NO. | Dry Weight in grams. | Amount $H_2O$ taken in grams | Amount $H_2O$ / Dry weight y | Time actually Occupied in hydration in hours. t. | y / t |
|---|---|---|---|---|---|
| 26 .3.1 | 1.307 | 1.331 | 1.019 | 41 | .0248 |
| 34 .2.2 | 1.917 | 1.535 | 1.283 | 18 | .0802 |
| 34A.1.2 | 1.070 | 1.285 | 1.200 | 14 | .0857 |
| 37A.2.1 | 1.040 | 2.699 | 2.598 | 53 | .0490 |
| 34 .2.1 | 1.033 | 1.427 | 1.382 | 20 | .0768 |
| 37A.1.1 | 1.021 | 2.215 | 2.170 | 46 | .0472 |
| 34A.1.1 | 1.000 | 1.226 | 1.226 | 13 | .0943 |
| 26 .3.2 | .920 | .875 | .950 | 33 | .0288 |
| 29 .3.1 | .920 | 1.255 | 1.365 | 26 | .0525 |
| 37A.2.2 | .914 | 2.157 | 2.360 | 15 | .1574 |
| 37A.1.2 | .865 | 1.760 | 2.035 | 22 | .0925 |
| 26A.1.1 | .777 | 1.129 | 1.453 | 75 | .0194 |
| 29 .4.2 | .754 | 1.228 | 1.630 | 21 | .0776 |
| 34 .2.3 | .705 | .845 | 1.199 | 28 | .0545 |
| 27 .3.2 | .674 | .851 | 1.263 | 22 | .0574 |
| 37A.2.3 | .653 | 1.847 | 2.827 | 21 | .1346 |
| 29 .4.1 | .573 | 1.122 | 1.960 | 21 | .0934 |
| 27 .1.1 | .518 | .653 | 1.261 | 28 | .0450 |
| 27 .3.1 | .271 | .784 | 2.894 | 27 | .1072 |
| 29 .3.2 | .221 | .653 | 2.955 | 22 | .1344 |

TABLE Vb. Hydration figures for "seeds in pod".

| Seed No. | Dry Weight Grams | Amount $H_2O$ taken in Grams. | Amount of $H_2O$ / Dry weight = y | Time actually occupied in hydration. = t Hours. | $\frac{y}{t}$ |
|---|---|---|---|---|---|
| 12 .1.3 | 2.340 | 2.652 | 1.134 | 44 | .0257 |
| 12 .1.1 | 2.196 | 2.383 | 1.085 | 42 | .0258 |
| 12 .7.1 | 2.180 | 1.940 | .913 | 14 | .0652 |
| 12 .1.2 | 2.056 | 2.419 | 1.176 | 61 | .0192 |
| 12 .3.2 | 1.948 | 1.772 | .910 | 30 | .0303 |
| 12 .1.4 | 1.735 | 2.274 | 1.310 | 79 | .0165 |
| 12 .4.1 | 1.638 | 2.187 | 1.334 | 47 | .0283 |
| 12 .3.3 | 1.632 | 1.991 | 1.220 | 51 | .0239 |
| 20A.1.3 | 1.611 | 2.089 | 1.298 | 33 | .0393 |
| 20A.1.2 | 1.480 | 2.099 | 1.420 | 51 | .0278 |
| 20A.1.4 | 1.476 | 1.833 | 1.241 | 79 | .0157 |
| 20A.2.2 | 1.406 | 1.697 | 1.206 | 24 | .0503 |
| 20A.2.1 | 1.344 | 1.620 | 1.205 | 30 | .0402 |
| 12 .4.2 | 1.293 | 1.446 | 1.120 | 47 | .0238 |
| 23A.1.1 | 1.167 | 1.516 | 1.300 | 22 | .0591 |
| 23A.2.2 | 1.106 | 1.758 | 1.590 | 28 | .0568 |
| 23A.2.3 | 1.077 | 1.445 | 1.341 | 22 | .0610 |
| 12 .4.3 | 1.075 | 1.332 | 1.239 | 49 | .0259 |
| 23A.2.1 | 1.024 | 1.634 | 1.596 | 46 | .0347 |
| 23A.1.3 | .903 | 1.259 | 1.394 | 26 | .0536 |
| 23A.1.2 | .816 | 1.195 | 1.465 | 31 | .0472 |
| 20A.1.1 | .780 | .975 | 1.250 | 28 | .0446 |
| 12.3.1 | .745 | .831 | 1.115 | 33 | .0338 |

TABLE Vc. Hydration figures for "seeds in pod on plant"

| Seed No. | Dry weight Grams | Amount of $H_2O$ taken in. Grams. | Amount of $H_2O$ / Dry weight y | Time actually occupied in hydration. = t Hours. | $\frac{y}{t}$ |
|---|---|---|---|---|---|
| 11 .4.1 | 2.134 | 2.615 | 1.225 | 49 | .0250 |
| 11 .5.1 | 1.954 | 2.320 | 1.178 | 44 | .027 |
| 11 .5.2 | 1.943 | 2.334 | 1.201 | 44 | .027 |
| 7B.2.1 | 1.508 | 1.949 | 1.292 | 28 | .046 |
| 10 .3.2 | 1.410 | 1.583 | 1.122 | 26 | .043 |
| 10 .3.3 | 1.353 | 1.489 | 1.110 | 25 | .044 |
| 10 .3.1 | 1.297 | 1.473 | 1.136 | 24 | .047 |
| 10 .2.1 | 1.210 | 1.330 | 1.100 | 23 | .048 |
| 10 .2.2 | 1.192 | 1.312 | 1.100 | 42 | .026 |
| 11* .3.2 | 1.175 | 1.345 | 1.145 | 25 | .046 |
| 11 .4.4 | 1.170 | 1.185 | 1.613 | 30 | .034 |
| 11* .3.1 | 1.100 | 1.122 | 1.020 | 25 | .042 |
| 11 .5.3 | 1.090 | 1.199 | 1.100 | 31 | .035 |
| 11* .4.2 | 1.068 | 1.352 | 1.266 | 17 | .074 |
| 11* .4.1 | 1.032 | 1.302 | 1.263 | 42 | .030 |
| 7B.2.2 | .956 | 1.257 | 1.315 | 24 | .055 |
| 11* .4.3 | .940 | 1.183 | 1.259 | 20 | .063 |
| 11 .4.2 | .820 | 1.078 | 1.315 | 48 | .027 |
| 4 .3.1 | .700 | 1.171 | 1.674 | 16 | .104 |
| 4 .3.2 | .284 | .937 | 3.300 | 44 | .075 |

two classes. In point of fact the mean dry weight of the "shelled seeds" is .814 grams, as compared to 1.436 grams for the "seeds in pod" group and 1.227 grams for the "seeds in pod on plant" group. Statistically the difference between the means of the second and third groups is not significant, while the difference between the means of the first and second, and first and third groups is significant. Two explanations for this difference may be put forward. In the first place it may be said that the seeds stored in the pod and those stored in the pod attached to the plant, have withdrawn plastic materials from the drying tissues surrounding them and have thus become heavier than those which had been separated from the parent tissues. Secondly it may be argued that the "shelled seeds" having been directly exposed to the storage conditions have dried out more thoroughly than the other two groups of seeds which were enclosed in their pods throughout the storage period. The answer as to which of these two explanations is the more probable is to be found in figures of Table VI. The average percentage moisture content of the air dry "shelled seeds" is 7.31% as compared to 7.74% for "seeds in pod" and 7.70% for "seeds in pod on plant." Roughly then, there is .4% less moisture in the shelled seeds than in the other two classes, which obviously cannot account for the very much bigger differences in dry weights. It is probable therefore that the

TABLE VI. Moisture content of seeds from the three storage classes.

| Seed No | Storage treatment | Dry Wt. grams. | Wt. after heating grams. | Loss in Wt. grams. | Percentage loss of dry weight. |
|---|---|---|---|---|---|
| 32.13.1 ) | | .263 | .225 | .038 | 6.92 |
| 32.13.2 ) | shelled | .259 | .221 | .038 | 6.81 |
| 30.11.1 ) | seeds. | 1.404 | 1.229 | .175 | 8.03 |
| 30. 4.1 ) | | .599 | .519 | .080 | 7.49 |
| 22.9.1 ) | | 1.539 | 1.347 | .192 | 8.02 |
| 22.9.2 ) | seeds in | .879 | .764 | .115 | 7.65 |
| 17.5.1 ) | pod. | .812 | .708 | .104 | 7.81 |
| 17.8.1 ) | | .667 | .578 | .089 | 7.5 |
| 9 .1.1 ) | | 1.085 | .946 | .139 | 7.8 |
| 3 .8.1 ) | In pod on | .806 | .699 | .107 | 7.53 |
| 3 .8.3 ) | plant | .754 | .657 | .097 | 7.77 |

lower mean dry weight of "shelled seeds" as compared to seeds "in pod" and "in pod on plant", is due to increase in weight of the latter two classes during storage as a result of the continued flow of plastic materials into the seeds from the parent tissues.

The time occupied in complete hydration by "shelled seeds" is lower than it is in either "seeds in pod" or "seeds in pod on plant." There are only 5 seeds with a hydration time of more than 30 hours in the shelled class while there are 16 in the "in pod" class and 9 in the "in pod on plant" class. The mean times for each of these classes in the same order is 27.8 hours, 42.5 hours, and 33 hours. It might at first be thought that since these means have the same relation to each other as the means for dry seed weight, the two figures were in some way correlated. Table VII shows that although a slight positive correlation does exist between dry weight of a seed and the time taken for it to hydrate completely, it is not significant.

An interesting relationship exists between the weight of a seed and the amount of water taken in by it. The relationship is apparent in the two unshelled classes but not in the shelled class. The apparent contradiction in the "shelled seeds" is explainable if we refer back to an observation made on certain seeds during their hydration. As hydration of the seeds of plant 37 a progressed it was noticed that the water in which each seed was

soaking, became more and more syrupy as time went on. A drop of this syrupy water was examined under the high power of the microscope and compared to a drop of water surrounding the seed of another plant. The syrupy water contained enormous numbers of medium sized rod bacteria while the control had none. A glance at the figures of Table Va will show that the seeds of plant 37a were abnormal in hydration, and we are forced to the conclusion that the presence of the bacteria was responsible for the abnormality, and this is further supported by the fact that all the seeds of plant 37a decayed rapidly and did not germinate.

Excluding therefore the seeds of this particular plant in the shelled class, a statistical correlation between seed weight and water intake of +.773 (see Table Vii) is found. For "seeds in pod" and "seeds in pod on plant" the same correlation is +.893 and +.943 respectively. In other words the amount of water taken in by a seed is proportional to its dry weight. The bigger the seed the more water it will absorb. From the above correlation figures it is manifest however, that the proportionality, though significant, is not so good in the shelled class as it is in the other two classes. The correlation figures between air dry seed weight and water intake per unit of dry matter (Table VII) provides the explanation. The correlation for "shelled seeds" is -.793, for "seeds in pod" -.571, and for "seeds in

TABLE VII. Correlation figures between figures of each of the three storage classes.

| Type of Storage | r for Wt. and $H_2O$ intake | r for Wt. and $\frac{H_2O}{Wt.}$ | r for Wt. and $\frac{y}{t}$ | r for Wt and t. | No of seeds in group. |
|---|---|---|---|---|---|
| Shelled seeds | +.773 | -.793 | - | - | 15 |
| Seeds in pod | +.893 | -.571 | -.356 | +.253 | 23 |
| Seeds in pod on plant. | +.943 | -.541 | - | - | 20 |

pod on plant" -.541. All three figures are significant, and from them the conclusion is justified that the smaller the seed the higher is the water intake per unit of dry matter. Moreover we note that whereas the correlation between weight and water intake for "shelled seeds" was the lowest that between weight and water intake per unit of dry matter is the highest of the three classes. Couple this observation, with the fact already stated that the mean of the dry weights of the "shelled seeds" is lower than the other two classes, and we come to the conclusion that the large number of small seeds in the "shelled seeds" class is responsible for the inferior proportionality between weight and water intake in this class as compared to the two unshelled classes. The answer to the question why the small seeds should absorb more water per unit of dry matter than big seeds, is however not so easy to explain. At this stage two possibilities may be put forward. In the first place it is possible that the greater water content of the heavier air dry seeds (c.f. Table VI) is in some way responsible for the comparatively smaller intake per unit of dry matter, the reverse holding for small seeds. Alternatively it may be said that the heavy seeds are more mature than the light seeds and that therefore the colloids of the two types are different. This latter view is given a measure of support by the fact that the small seeds in any of the three classes

contain less moisture in the air dry condition than the large, which would indicate that the water holding capacity of the respective colloids is different, and from which would follow that the colloids themselves are in some respects different. As against this however it must be mentioned, that the mass to surface area ratio in small seeds is higher than it is in big seeds, and that the greater drying out of small seeds may be due to this greater ratio and not to the smaller water holding capacity of the colloid. Though the data presented is insufficient to explain the reason, the fact that the small seeds take up a greater amount of water per unit of dry matter remains, and constitutes one of the factors which go to make up the tremendous variations exhibited between seed and seed.

The column headed y/t represents the amount of water taken in by a seed per unit of air dry matter per unit of time. The time (t) is not the period that the seed was actually in the water, but the time the seed was actually hydrating that is from the first increase in weight to full hydration. This figure was calculated for each seed so as to give an indication of the efficiency of the seed. Statistical treatment of this column of figures for each of the three classes shows that it has no significance. The reason for this will become more apparent later, when further work is discussed.

b) The degree of variation of seeds in the same pod and on the same plant.

The figures for seeds of a number of pods which will be used in this section are given in Table VIII. These seeds are all those of Tables Vb and Vc (the two "unshelled classes"), less those which were in some way abnormal in hydration. In addition two normal seeds have been eliminated since each of these originated, in a two seeded pod together with an abnormal seed. The seeds of the "shelled class" (Table Va) have not been used here since it has already been shown that these are significantly different in some important respects from the two unshelled classes due, it is suggested to their removal from the tissues of the parent plant. For the purpose to which these figures are to be put - where seeds of one pod will be compared with seeds of other pods, and where seeds of one plant will be compared with seeds of other plants - it is necessary that only uniform material be considered. It has been shown that the seeds of the two unshelled classes are not significantly different in their general reaction and their combined use here is therefore justified.

The object of this section is to determine the difference in degree of variation of seeds within a pod and between pods and of seeds on a plant and between plants. For this purpose it was decided to employ the statistical method of analysis of variance described by Tippett (1931 p. 96 et seq.) Essentially

TABLE VIII. The degree of variation of seeds of the same pod and of the same plant.

| Seed No | Dry Wt. Grams | Water intake Grams | $\frac{H_2O \text{ intake}}{\text{Dry Wt.}} = y$ | Corrected Time to compl. hyd. = t Hours. | $\frac{y}{t}$ |
|---|---|---|---|---|---|
| 12.1.1 ) | 2.196 | 2.383 | 1.085 | 42 | .0258 |
| 12.1.2 ) | 2.056 | 2.419 | 1.176 | 61 | .0192 |
| 12.1.3 ) | 2.340 | 2.652 | 1.134 | 44 | .0257 |
| 12.1.4 ) | 1.735 | 2.274 | 1.310 | 79 | .0165 |
| 12.3.1 ) | .745 | .831 | 1.115 | 33 | .0338 |
| 12.3.2 ) | 1.948 | 1.772 | .910 | 30 | .0303 |
| 12.3.3 ) | 1.632 | 1.991 | 1.220 | 51 | .0239 |
| 12.4.1 ) | 1.638 | 2.187 | 1.334 | 47 | .0283 |
| 12.4.2 ) | 1.293 | 1.446 | 1.120 | 47 | .0238 |
| 12.4.3 ) | 1.075 | 1.332 | 1.239 | 49 | .259 |
| 20A.1.1) | .780 | .975 | 1.250 | 28 | .0446 |
| 20A.1.2) | 1.480 | 2099 | 1.420 | 51 | .0278 |
| 20A.1.3) | 1.611 | 2.089 | 1.298 | 33 | .0393 |
| 20A.1.4) | 1.476 | 1.833 | 1.241 | 79 | .0157 |
| 20A.2.1) | 1.344 | 1.620 | 1.205 | 30 | .0402 |
| 20A.2.2) | 1.406 | 1.697 | 1.206 | 24 | .0503 |

TABLE VIII. (contd.)

| Seed No | Dry Wt. Grams. | Water intake Grams. | $\frac{H_2O \text{ intake}}{\text{Dry Wt.}} = y$ | Corrected Time to compl. hyd.= t Hours. | $\frac{y}{t}$ |
|---|---|---|---|---|---|
| 23A.1.1 ) | 1.167 | 1.516 | 1.300 | 22 | .0591 |
| 23A.1.2 ) | .816 | 1.195 | 1.465 | 31 | .0472 |
| 23A.1.3 ) | .903 | 1.259 | 1.394 | 26 | .0536 |
| 23A.2.1 ) | 1.024 | 1.634 | 1.596 | 46 | .0347 |
| 23A.2.2 ) | 1.106 | 1.758 | 1.590 | 28 | .0568 |
| 23A.2.3 ) | 1.077 | 1.445 | 1.341 | 22 | .0610 |
| 11 .4.1 ) | 2.134 | 2.615 | 1.225 | 49 | .0250 |
| 11 .4.2 ) | .820 | 1.078 | 1.315 | 48 | .0270 |
| 11 .4.4 ) | 1.170 | 1.185 | 1.013 | 30 | .0340 |
| 11 .5.1 ) | 1.954 | 2.320 | 1.178 | 44 | .0270 |
| 11 .5.2 ) | 1.943 | 2.334 | 1.203 | 44 | .0270 |
| 11 .5.3 ) | 1.090 | 1.199 | 1.100 | 31 | .0350. |

the method consists of computing the mean variances of the two classes (i.e. "within pod" and "between pods") and obtaining from these a figure "z" which is a measure of the significance of the difference between the two mean variances. Treatment of the figures of dry seed weight by this method gives a "z" of .4231; for the water intake figures .3636; and for the water intake per unit of dry weight .7388. Tables of "z" values given by Fisher (1932) show that for degrees of freedom of 12 and 25, as in this case, a "z" of .3862 lies on the 5 per cent point, and a "z" of .5481 lies on the one per cent point. According to Tippett and Fisher a value of "z" which lies beyond the 5 or 1 per cent point of Fisher's tables may be considered as significant. It is justifiable to conclude therefore, that the difference between the estimates of variance of water intake per unit of dry weight for seeds within the pod and for seeds between pods is very significant. The "z" figure for dry seed weights is also significant, though not to the same extent as the "z" for water intake per unit of dry weight. The figure .3636 obtained for water intake falls below the 5 per cent point and is therefore not completely significant, but on the other hand it must not be supposed that the two variances are equivalent. If this had been the case a "z" of zero would have been obtained. The "z" actually obtained is only .0226 below that which lies on the 5 per cent point.

The interpretation of these results is that there is less variation between seeds of the same pod than between seeds of different pods. In other words individuality is not so pronounced if only seeds from the same pod are compared. The same statement is true if the figures for y/t (amount of water absorbed per unit of dry weight per unit of time) are considered. Here a "z" of .7125 is obtained. The figures for t (corrected time to complete hydration) however, give a "z" of only .3485. The difference in mean variance of t of the "within pod" and "between pod" groups is therefore of doubtful significance.

To bring out the difference in mean variance of the seeds on one plant and between plants, the figures of Table VIII were recalculated. The method of statistical analysis is exactly the same as that previously used except that instead of "within pod" and "between pod" we have two new groups "on plant" and "between plants". Each of the five columns of figures of Table VIII were treated on this new basis and a "z" value for each column obtained. This "z" value indicated the amount of difference there is between the mean variances of the two groups - "on plant" and "between plants." The "z" values for each of the five columns of figures thus obtained are given in Table IX together with the corresponding "z" values obtained for the "within pod" and "between pod" groups. On the same table the degrees of freedom and Fisher's "z" values for the 1 and 5 per cent points

TABLE IX. "z" for seeds "within pod" and "between pods" and for seeds "on plant" and "between plants."

| "z" for | "Between pods" and "within pods". | "On plant" and "between plants." |
|---|---|---|
| Dry Seed Wt. | .4231 | .5447 |
| Water intake | .3636 | .3694 |
| Water intake/Dry Wt. = y | .7388 | 1.0008 |
| Time to complete hydration = t | .3485 | .2676 |
| y/t | .7125 | 1.0764 |

| | | |
|---|---|---|
| Degrees of freedom | $n_1 = 12, n_2 = 25$. | $n_1 = 5, n_2 = 32$. |
| Fisher's 1 per cent point | .5481 | .6540 |
| Fisher's 5 per cent point | .3862 | .4648 |

for the same degrees of freedom are given for each of the two sets of "z" values calculated.

The degrees of freedom for the "on plant" group is 32, and for the "between plant" group,5. Fisher's "z" tables show that for these degrees of freedom a "z" value of .6540 lies on the 1 per cent point of distribution and .4648 lies on the 5 per cent point. Examination of the "z" values obtained for the "on plant" and "between plant" groups given in table IX show that the "z" for dry seed weight is greater than that which lies on the 5 per cent point, while the "z" values for water intake per unit of dry weight and for y/t (water intake per unit of dry weight per unit of time) are considerably higher than that which lies on the 1 per cent point of distribution. The logical inference is that on the basis of figures for dry seed weight, water intake per unit of dry weight, and y/t there is less variation between seeds of the one plant than between seeds of different plants. This is not the case if the figures for water intake and corrected time to complete hydration are considered. The "z" values in these two cases are below that for the 5 per cent point.

These results bring us to the conclusion that the variation is less between seeds of the same pod than between seeds of different pods, and less between seeds of one plant than between seeds of different plants. The fact that rarely more than two pods occur on a plant precludes comparisons of mean

variance of seeds of one pod with seeds of other pods on the same plant. It is doubtful however if there is any significant difference in the mean variance of seeds within the pod and the mean variance of all the seeds on the same plant. The "z" values obtained for "within pod" and "between pod" take into account not only the variation existing between pod and pod on the same plant, but also that existing between plant and plant since pods were compared with pods irrespective of the plants from which these arose. If therefore there had been a significant difference in the mean variance of seeds within the pod and the mean variance of all seeds on the same plant, the "z" values of "between pods" and "within pods" would be higher than the corresponding values for "on plant" and "between plants". In fact they are lower, and hence we may reasonably assume that the variation of seeds within a pod is equivalent to the variation of all seeds on the same plant.

The bearing of these findings on the discussion of individuality is of importance in that they show clearly that even in a commercial pure line there is considerable variation in the water intake characteristics of seeds from different plants. The point is clearly seen in the reduction of individuality brought about by comparing seeds from the same plant. The two most important significant "z" values are those for dry seed weight and the y/t coefficient

(water intake per unit of dry weight per unit of time.) The first indicates a quantitative difference in seeds of different plants, and the second indicates a qualitative difference in seeds of different plants. It is not impossible that the qualitive difference is a result of the quantitative difference due either to the greater dryness of the seed having caused changes in the structure of the seed colloids or to the immaturity of the small seed as compared to the big seed. These two possibilities have been noted earlier in this chapter. On the other hand it must be remembered that a genetical mechanism may quite well underlie the quantitative and qualitative differences between seeds of different plants. Whatever may be the cause of causes of these differencies, the matter is of prime importance for future studies.

c. The relationship between locus of first hydration, rate of initial intake of water, time of micropyle opening and time of complete hydration.

In Table X against each seed there are noted three figures and one observation. Each of these columns requires some explanation. The figure in the first column are corrected times for micropyle opening. The micropyle is considered open when gentle pressure on either side of the seed forces water out of the micropyle. Each figure given is arrived at by subtracting from the observed time of micropyle opening of the seed in question, the interval which elapsed from the commencement of the experiment during which no increase in the weight of the seed took place. The second column for complete hydration time involves the same correction. It is the difference between the observed time of complete hydration and the initial interval during which no increase in weight took place. The third column gives the rate of water intake per hour for the first five hours of weight increase in grams per hour. This figure also then has only been arrived at after first making the same correction. In this way all the figures in each of the three columns have been put on exactly the same basis. As soon as a seed is placed in water there is an increase in weight in the first hour not exceeding .005 grams, but thereafter there follows a period varying with different seeds during which no further increase

TABLE X. Influence of the locus of commencement of hydration on the rate of initial water intake, the relationship between rate of water intake and micropyle opening, and the relationship between time of micropyle opening and time of complete hydration.

| Seeds No | Corrected time of micropyle opening in hours. | Corrected time to complete hydration in hours. | Rate of $H_2O$ intake for the 1st 5 hours of hydration grs./hour | Locus of commencement of hydration. |
|---|---|---|---|---|
| 37A .1.1 | 16 | 46 | .0056 | Strophiole. |
| 1.2 | 4 | 22 | .1958 | Hilar. |
| 37A .2.1 | 8 | 53 | .0044 | Periphery. |
| 2.2 | 3 | 15 | .3058 | Hilar. |
| 2.3 | 2 | 21 | .0934 | Hilar. |
| 34A .1.1 | 5 | 13 | .1682 | Cotyl. face. |
| 1.2 | 6 | 14 | .0088 | Ov. embryo. |
| 34 2.1 | 6 | 20 | .0456 | Periphery. |
| 2.2 | 10 | 18 | .0396 | " |
| 2.3 | X | 22 | .0130 | Ov. embryo. |
| 27 .3.1 | X | 27 | .0072 | Hilar |
| 3.2 | 11 | 22 | .0016 | Over embryo. |
| 27 .1.1 | 9 | 28 | .0096 | Hilar. |
| 26 .3.1 | - | 41 | .0070 | Periphery. |
| 3.2 | - | 36 | - | " |
| 26A .1.1 | - | 75 | - | - |
| 29 .3.1 | 14 | 26 | .0320 | Periphery. |
| 3.2 | X | 22 | .0144 | Hilar. |
| 29 .4.1 | 6 | 21 | .0594 | Hilar. |
| 4.2 | 8 | 21 | .0254 | Stroph. and ov. embryo. |
| 23A .2.1 | 12 | 46 | .0108 | Periphery. |
| 2.2 | 15 | 28 | .0078 | " |
| 2.3 | 9 | 22 | .0132 | Ov. embryo. |
| 2.4 | 8 | 22 | .0178 | Periphery. |
| 23A .1.1 | 13 | 22 | .0018 | Periphery. |
| 1.2 | 9 | 31 | .0108 | Hilar. |
| 1.3 | 9 | 26 | .0108 | " |
| 20A .1.1 | 10 | 28 | .0084 | Strophiole. |
| 1.2 | - | 51 | - | - |
| 1.3 | - | 33 | - | - |
| 1.4 | - | 79 | - | - |
| 20A .2.1 | 13 | 30 | .0024 | Periphery. |
| 2.2 | 12 | 24 | .0048 | Strophiole. |
| 12 .7.1 | 5 | 14 | .1520 | Simultaneously all over. |
| 12 .1.1 | - | 42 | .0034 | Cotyl. face. |
| 1.2 | 43 | 61 | - | Strophiole. |
| 1.3 | 22 | 44 | .0044 | Cotyl. face. |
| 1.4 | 53 | 79 | - | - |
| 12 .3.1 | X | 33 | .0066 | Hilar |
| 3.2 | 23 | 30 | .0038 | Cotyl. face. |

TABLE X. (contd.)

| Seeds No | Corrected time of micropyle opening in hours. | Corrected time to complete hydration in hours. | Rate of $H_2O$ intake for the 1st 5 hours of hydration grs./hour | Locus of commencement of hydration. |
|---|---|---|---|---|
| 3.3 | 36 | 51 | - | - |
| 12 .4.1 | 27 | 47 | .0074 | Cotyledon face. |
| 4.2 | 12 | 47 | .0102 | Hilar. |
| 4.3 | 15 | 49 | .0120 | - |
| 4 .3.1 | 7 | 16 | .0252 | Periphery. |
| 3.2 | X | 44 | .0060 | Hilar. |
| 11* .3.1 | 11 | 25 | .0186 | Periphery. |
| 3.2 | 6 | 25 | .0322 | Hilar. |
| 11* .4.1 | 18 | 42 | .0076 | Ove. emb. |
| 4.2 | 7 | 17 | .0132 | Hilar. |
| 4.3 | 8 | 20 | .0084 | " |
| 11 .4.1 | 25 | 49 | .0036 | Cotyl. face. |
| 4.2 | 6 | 48 | .0144 | Hilar. |
| 4.4 | 13 | 30 | .0048 | Hilar. |
| 11 .5.1 | - | 44 | .0026 | Hilar. |
| 5.2 | 20 | 44 | .0046 | " |
| 5.3 | 11 | 31 | .0138 | " |
| 10 .2.1 | 6 | 23 | .0260 | Hilar. |
| 2.2 | 22 | 42 | .0066 | Strophiole |
| 10 .3.1 | 6 | 24 | .0204 | Hilar. |
| 3.2 | 9 | 26 | .0260 | Periphery. |
| 3.3 | 11 | 25 | .0224 | " |
| 7 .2.1 | 9 | 28 | .0094 | Hilar. |
| 2.2 | 6 | 24 | .0316 | Hilar and Periphery. |

takes place. This increase in the first hour is assumed to be due to the penetration of water into cracks and crevices of the seed coat where surface drying will not be effective. This slight initial increase has therefore been ignored.

The meaning of the observations in the column entitled "Locus of commencement of hydration" requires some definition. "Hilar " signifies that the testa wrinkled (taken as evidence of hydration) first at either end or on either side of the hilum. "Periphery" means that wrinkling commenced somewhere between the strophiole and the embryo on the non-hilar periphery of the seed. "Strophiole" implies first wrinkling at the strophiole, and "Over embryo" first wrinkling in that portion of the testa immediately over the embryo. If wrinkling commenced on either side of the seed the phrase "Cotyledon face" is used.

The 64 seeds of Tables Va, Vb, and Vc, are included in Table X. All the seeds noted in Table X are not suitable for the demonstration of some of the points which follow, It is essential therefore to define the material used before making each point.

For the first conclusion attention must be focussed on pods which conform to the following requirements. Two seeds or more, some of which but not all having a hilar locus of hydration, and further the figures for rate of initial intake must be known for two contrasted seeds in respect of locus of first hydration. There are 14 pods in Table X

which meet these requirements. Comparing the seeds within these pods it is evident that a high rate of initial water intake is associated with a Hilar locus of first hydration. Of the 14 pods which are suitable, 11 agree and three disagree with this statement. Expressed as a percentage there is 78% agreement. These must therefore be some significance in the conclusion, and the explanation is to be found in the anatomical structure of the seed coat. When describing the anatomy of the testa it was evident that there is a very much larger mass of tissue in cross section in the hilar region than there is in any other part of the testa. Over the greater part of the seed the testa consists of three main layers of more or less uniform depth except in the hilar region. In this region there is no second or hour glass layer but there is instead a very absorbent and much bigger tissue, the "tracheid island". In addition there is a double palisade layer running along the whole length of the hilum, a large pad of curiously star-shaped parenchyma surrounding the tracheid island and projecting from the nutrient layer there is the bulky parenchymatous tissue which surrounds the radicle. When the tissue below the hilum becomes hydrated it swells up absorbing a great deal of water. Since there is more of this tissue in the hilar region the total absorbent capacity per unit surface area of the testa in this region will be greater than it is in any other part of the testa.

For demonstration of the next point it is necessary to confine attention to pods conforming to the same conditions as stipulated in the last case except that the figures for micropyle opening must be known instead of the figures for rate of water intake. There are ten pods which meet these requirements and without exception the seeds in each of these pods show that a seed with a locus of first hydration in the vicinity of the hilum is quicker in opening its micropyle than seeds with a locus of hydration other than around the micropyle. This association between micropyle opening and locus of hydration confirms observations frequently made during experimentation that micropyle opening is conditioned by the hydration of the tissues immediately surrounding it. The explanation of how the micropyle opens as a result of hydration is not known with certainty. If it is accepted that the micropyle is a definite channel extending from the exterior to the interior of the seed coat, then the explanation might be that the channel remains closed until through hydration of the tissues in its immediate vicinity the walls of the channel separate thus making a clear passage for the entry of water.

It would be expected that since there is a relationship between rate of initial water intake and locus of commencement of hydration, and between the latter and time of micropyle opening, there should also be a relationship between rate of initial intake

and micropyle opening. This is in fact the case. Using all seeds in Table X, 47 in number for which both figures are known, a negative correlation of .480 is obtained. Since this figure is greater than the value which may occur on 1% occasions as a matter of chance by random sampling from an uncorrelated population, it may be considered as significant. The interpretation of this negative correlation is that the higher the initial rate of water intake the shorter the time for micropyle opening which is exactly the same conclusion as that which is arrived at by combining the observations that a hilar locus of commencement of hydration is related to (a) a high initial rate of water intake, and (b) a short time of micropyle opening.

Finally using all those seeds in Table X which are complete in respect of the figures for corrected time of micropyle opening and corrected time to complete hydration, and treating the two columns of figures statistically, a positive correlation of .832 for 50 seeds is arrived at. In other words the quicker the micropyle opens the shorter will be the time for complete hydration. This is understandable since once the cotyledons are directly in contact with water there is nothing to hinder their complete hydration in minimum time. Though it would not be true to say that there is no variation in the time of complete hydration of paired cotyledons, it can with certainty be stated that this variation is not of the

same order as that which pertains to whole seeds.

Since a Hilar locus of hydration implies a high initial rate of intake of water and a short time to micropyle opening, and since the latter implies a short time to complete hydration, it follows that a hilar locus of hydration is associated with a short time to complete hydration. Consideration of these pods, the seeds of which numbering two or more are contrasting in that some but not all have a hilar locus of hydration, will show that of the fourteen pods which are suitable for this purpose, ten confirm the statement that a hilar locus of commencement of hydration is associated with a short time of complete hydration. There is just over 70% of the pods in agreement.

Though no hard and fast rule can be made, it is reasonable to summarize the conclusions so far arrived at in this section by saying that prerequisites of a short time for complete hydration are a short time to micropyle opening and its corollary a first locus of hydration in the vicinity of the micropyle. It must however be borne in mind that the time to complete hydration referred to is only that time during which actual increase in weight of the seed is taking place. No account whatever is taken of that period of time following commencement of soaking during which no increase in weight takes place. This latter period is one which varies tremendously from seed to seed, and the reason for its variation or for

its existence at all is unknown. Nevertheless the conclusions so far reached, though limited, are valuable in that they partially account for the individuality of the time factor in the hydration of the seed.

A further point which comes out from consideration of the locus of commencement of hydration of seeds is that seeds of the same pod appear to commence hydration in one of two places only. The loci of first hydration of seeds of ten three or more seeded pods are given in Table XI. Examination of this Table will show that the above statement is upheld. Coupled with the relationships established above it is obvious that this finding has great significance. It implies that the actual time occupied by a seed in complete hydration is determined not during storage but either in development of the seed in the pod or even further back at fertilization. If the explanation is to be sought during the development of the seeds in the pod it must be assumed that the differences are produced purely by physical differences exerted on different seeds in the pod. It is conceivable that pressure in a certain place on one seed and not another will produce the patterning evident at the commencement of hydration. This however does not explain the existence of only two patterns in any one pod. As an alternative explanation a genetic pattern factor complex may be postulated.

TABLE XI. Variation in locus of hydration of seeds in the same pod.

| Seeds No | Locus of hydration. |
|---|---|
| 11 .5.1 | Hilar. |
| 11 .5.2 | Hilar. |
| 5.3 | Hilar. |
| 11 .4.1 | Cotyledon face. |
| 4.2 | Hilar. |
| 4.4 | Hilar. |
| 10 .3.1 | Hilar. |
| 3.2 | Periphery. |
| 3.3 | Periphery. |
| 11* .4.1 | Over embryo. |
| 4.2 | Hilar. |
| 4.3 | Hilar. |
| 37A .2.1 | Periphery. |
| 2.2 | Hilar. |
| 2.3 | Hilar. |
| 12 .4.1 | Cotyledon face. |
| 4.2 | Hilar. |
| 4.3 | Hilar. |
| 12 .1.1 | Cotyledon face. |
| 1.2 | Strophiole. |
| 1.3 | Cotyledon face. |
| 1.4 | - |
| 23A .1.1 | Periphery. |
| 1.2 | Hilar. |
| 1.3 | Hilar. |
| 23A .2.1 | Periphery. |
| 2.2 | Periphery. |
| 2.3 | Over embryo. |
| 2.4 | Periphery. |
| 34 .2.1 | Periphery |
| 2.2 | Periphery. |
| 2.3 | Over embryo. |

d) Relationship of hydration idiosyncracies of a seed and the growth of the plant produced.

The abnormality of some seeds in not opening their micropyles and the predetermining influence of this must be referred to first before embarking on a general discussion on the subject of the title of this section.

In Table XII are noted all those abnormal seeds which do not open their micropyles, and for contrast the seeds from the same pods as well. Examination of the dry weights of these seeds will show that they are the lightest seeds in each of their pods. Apart from this character two of these seeds are otherwise normal and cannot be distinguished from other seeds of the same pods. The other three seeds however show very marked differences as compared to the normal seeds from the same pod. They are very light, have a very high water absorbed to weight ratio and further the seeds decay and do not germinate. It has already been shown that a light seed absorbs more water per unit of dry weight than a heavy seed, but the fact that these light and abnormal seeds do not germinate lends weight to the suggestion previously made that the reason for the greater absorption of small seeds is the physically and chemically different colloids of the smaller seed due to its immaturity.

In Table XIII are listed a representative collection of the seeds of Tables Va, Vb, and Vc. Four of the figures given for each seed are the same

TABLE XII. Relationships of seeds which are abnormal in that they do not open their micropyles.

| Seed No | Dry Wt. grams. | Water absorbed/ Dry Wt. | Corr. time to microp. open. Hours | Remakks. |
|---|---|---|---|---|
| 12 .3.1 | .745 | 1.115 | X | Germinated. |
| 3.2 | 1.948 | .910 | 23 | Germinated. |
| 3.3 | 1.632 | 1.220 | 36 | Germinated. |
| 34 .2.1 | 1.033 | 1.382 | 6 | Germinated. |
| 2.2 | 1.197 | 1.283 | 10 | Germinated. |
| 2.3 | .705 | 1.199 | X | Germinated. |
| 27 .3.1 | .271 | 2.894 | X | Seed Decayed. |
| 3.2 | .674 | 1.263 | 11 | Germinated. |
| 29 .3.1 | .920 | 1.365 | 14 | Germinated. |
| 3.2 | .221 | 2.955 | X | Seed Decayed. |
| 4 .3.1 | .700 | 1.674 | 7 | Germinated. |
| 3.2 | .284 | 3.300 | X | Seed Decayed. |

X = Micropyle never opened.

TABLE XIII. Relationship between hydration characteristics and growth of subsequent plant.

| Seed No | Dry Wt. grs. | $\frac{H_2O \text{ absorded}}{\text{Dry Wt.}} = y$ | Corrected time occupied in hydration = t hours. | $\frac{y}{t}$ | Days to Germination | Germ. to app. of plumule in days | Height of plant 30 days after germ. cms. |
|---|---|---|---|---|---|---|---|
| 34A.1.1 | 1.000 | 1.226 | 13 | .0943 | 11 | 22 | 8 |
| 1.2 | 1.070 | 1.200 | 14 | .0857 | 4 | 9 | 29 |
| 34 .2.1 | 1.033 | 1.382 | 20 | .0768 | 1 | 8 | 34 |
| 2.2 | 1.197 | 1.283 | 18 | .0802 | 3 | 11 | 21.5 |
| 2.3 | .705 | 1.199 | 22 | .0545 | 1 | 8 | 23.5 |
| 26 .3.1 | 1.307 | 1.019 | 41 | .0248 | 1 | 8 | 27.5 |
| 3.2 | .920 | .950 | 36 | .0288 | 2 | 12 | 14 |
| 23A.2.1 | 1.024 | 1.596 | 46 | .0347 | 2 | 16 | 22.5 |
| 2.2 | 1.106 | 1.590 | 28 | .0568 | 3 | 10 | 18.5 |
| 2.3 | 1.077 | 1.341 | 22 | .0610 | 2 | 8 | 10.5 |
| 23A.1.1 | 1.167 | 1.300 | 22 | .0591 | 2 | 8 | 20.5 |
| 1.2 | .816 | 1.465 | 31 | .0472 | 2 | 9 | 22.5 |
| 1.3 | .903 | 1.394 | 26 | .0536 | 2 | 9 | 17.5 |
| 20A.1.1 | .780 | 1.250 | 28 | .0446 | 1 | 7 | Died. |
| 1.2 | 1.480 | 1.420 | 51 | .0278 | 1 | 8 | 32.5 |
| 1.3 | 1.611 | 1.298 | 33 | .0393 | 2 | 10 | 34.5 |
| 1.4 | 1.476 | 1.241 | 79 | .0157 | 2 | 12 | 14.5 |
| 20A.2.1 | 1.344 | 1.205 | 30 | .0402 | 3 | 8 | 20.0 |
| 2.2 | 1.406 | 1.206 | 24 | .0503 | | | |
| 12 .1.1 | 2.196 | 1.085 | 42 | .0258 | 1 | 7 | 40 |
| 1.2 | 2.056 | 1.176 | 61 | .0192 | 1 | 9 | 44.5 |
| 1.3 | 2.340 | 1.134 | 44 | .0257 | 2 | 8 | 32.5 |
| 1.4 | 1.735 | 1.310 | 79 | .0165 | 1 | 20 | 2.5 |

TABLE XIII. (contd.)

| Seed No. | Dry Wt. grs. | $\frac{H_2O \text{ absorbed}}{\text{Dry Wt.}} = y$ | Corrected time occupied in hydration = t hours. | $\frac{y}{t}$ | Days to Germination | Germ. to app. of plumule in days. | Height of plant 30 days after germ. cms. |
|---|---|---|---|---|---|---|---|
| 12 .3.1 | .745 | 1.115 | 33 | .0338 | 1 | 9 | 28 |
| 3.2 | 1.948 | .910 | 30 | .0303 | 2 | 8 | 14 |
| 3.3 | 1.632 | 1.220 | 51 | .0239 | 1 | 8 | 35 |
| 12 .4.1 | 1.638 | 1.334 | 47 | .0283 | 1 | 8 | 28 |
| 4.2 | 1.293 | 1.120 | 47 | .0238 | 3 | 9 | 30.5 |
| 4.3 | 1.075 | 1.239 | 49 | .0259 | 3 | 18 | 14.3 |
| 11* .3.1 | 1.100 | 1.020 | 25 | .042 | 1 | 8 | 30.5 |
| .3.2 | 1.175 | 1.145 | 25 | .046 | 2 | 8 | 27.5 |
| 11* .4.1 | 1.032 | 1.263 | 42 | .030 | 4 | 21 | 9.0 |
| 4.2 | 1.068 | 1.266 | 17 | .074 | 2 | 8 | 23.5 |
| 4.3 | .940 | 1.259 | 20 | .063 | 1 | 8 | 25.5 |
| 11 .4.1 | 2.134 | 1.225 | 49 | .025 | 6 | 17 | 6.0 |
| 4.2 | .820 | 1.315 | 48 | .027 | 2 | 7 | 33.5 |
| 4.4 | 1.170 | 1.013 | 30 | .034 | 2 | 14 | 23.5 |
| 11 .5.1 | 1.954 | 1.178 | 44 | .027 | 2 | 8 | 33.5 |
| 5.2 | 1.943 | 1.201 | 44 | .027 | 3 | 9 | 39 |
| 5.3 | 1.090 | 1.100 | 31 | .035 | 44 | 13 | 8 |
| 10 .2.1 | 1.210 | 1.100 | 23 | .048 | 3 | 6 | 35.0 |
| 2.2 | 1.192 | 1.100 | 42 | .026 | 2 | 9 | 35.5 |
| 10 .3.1 | 1.297 | 1.136 | 24 | .047 | 2 | 7 | 29.0 |
| 3.2 | 1.410 | 1.122 | 26 | .043 | 2 | 9 | 20.5 |
| 3.3 | 1.353 | 1.110 | 25 | .044 | 2 | 6 | 35.5 |
| 7B .2.1 | 1.508 | 1.292 | 28 | .046 | 5 | 6 | 7.5 |
| 2.2 | .956 | 1.315 | 24 | .055 | 15 | 7 | |

as those given previously, and three of them relate to the growth of the plant produced by the seed. The first of the latter is the time in days taken by the seed to germinate from the time of planting to the time of protrusion of the radicle. The second gives the time in days the plumule took to appear at the surface of the soil from the date of germination. Finally the third column gives the height of the plant in centimetres thirty days after germination. This measurement was made from the surface of the soil to the highest point on the plant. It is not claimed that this figure is above criticism, it is merely claimed that the figures are broadly comparable. It is further realized that total height of plant does not give a true picture of the growth of the plant, particularly as the plants were grown in winter under very artificial conditions in a glass house.

Very careful examination of the figures tabulated has shown that there is no relationship between any of the figures for hydration of the seed and those for the seedling and plant subsequently produced. The figures for growth being admittedly crude a categorical statement is not warranted, but it might be said from data obtained in a preliminary experiment that the hydration idiosyncracies of a seed are not reflected in the initial growth stages of the plant it produces.

With regard to the relationship between germination time and growth characteristics of the plant, it may justly be stated broadly that the longer a seed takes

to germinate the longer it is before the plumule appears and the shorter the plant thirty days after germination. This will mostly clearly be seen by comparing a seed with a long germination time with a seed of the same pod having a short germination time.

## Summary of Conclusions.

I Seeds which are shelled before storage are significantly lighter than seeds stored in the pod or in the pod on the plant. This is due to increase in weight of the seeds of the latter two classes during storage as a result of the continued flow of plastic materials into the seeds from the parent tissues.

II While the total amount of water absorbed by any seed is in a measure proportional to the air dry weight of the seed the amount of water absorbed per unit of air dry weight is higher in small seeds than in large seeds. It is suggested that this greater potential water absorbing capacity of the dry matter of small seeds is connected with the facts that such seeds may be less mature than comparable large seeds giving a difference in quality of the dry matter, or that by reason of their surface to mass relationship they have dried out more after harvest and hence have a greater capacity for water after steeping.

III There is a greater degree of quantitative and qualitative similarity between seeds of the same plant than between seeds of different plants.

IV. A hilar locus of commencement of hydration is associated with a high rate of initial water intake and a short time to micropyle opening. It follows that micropyle opening is conditioned by the hydration of the tissues in its immediate vicinity.

V. A prerequisite of a short time to complete hydration is a short time to micropyle opening and its corollary a hilar locus of hydration. A high positive correlation exists between time of micropyle opening and complete hydration time. Both these figures are exclusive of the initial period of soaking during which no increase in the weight of the seed takes place.

VI. Seeds from the same pod commence to hydrate in one of two places only.

VII. Some very small seeds which do not open their micropyles have a very high water absorbed to

dry matter ratio and do not germinate. This gives weight to the suggestion already made that abnormality of small seeds is due to their immaturity.

VIII A slow germinating seed produces a slower growing plant than a quick germinating seed.

# Chapter. V

## Hysteresis of the seed colloids.

Contents.

## Introduction.

Gortner (1931) working with gelatine discs of known history found that the prehistory of a colloid has a pronounced effect on its hydration. It was thought probable that the same would hold for seeds, though it was realized that the two cases are not strictly parallel. Gelatine is a physically and chemically homogenous colloid whereas the seed is a complex of a number of colloid systems. We would expect therefore that the proof that hysteresis has a pronounced effect on hydration is fraught with greater difficulties in the case of the seed than it is in the case of gelatine.

It has long been known that storage conditions profoundly affect the germination of seeds, but little is known regarding the effect of these same storage conditions on the hydration of seeds! This latter question is of the greatest importance since it may throw some considerable light on some of the more important problems which confront the seed trade, such as "hard" and damaged seeds. A closely related problem is that of longevity of seeds. The literature abounds with observations of the germination of seeds as related to their age, and the difference which exists from one species to another in this respect. But no attempt is made to discover the causes of these differences. To do this it would be necessary to follow the physical and chemical changes and the resulting changes in physiological behaviour which

occur during the ageing process of each species.

It must here be emphasized that the experiments described in this chapter are exploratory in nature and any conclusions which are drawn are provisional.

Material and Method.

Seeds from eighty plants of Vicia Faba, were harvested on October 6th, 1934. The plants were grown in the open at the Royal Botanic Garden, Edinburgh, and were all of approximately the same age since they were all planted within a day or two of each other. Each seed was placed in a perforated envelope and placed in one of ten treatments after recording its plant of origin, the pod on that plant and its particular position in that pod. Prior to harvesting the seeds estimated to be available by a preliminary count, were distributed through the ten treatments so that effects due to difference in position of pods from one plant to the other and difference in position of seeds in any one pod would be eliminated. The ten treatments above mentioned are as follows:-

| | | | |
|---|---|---|---|
| I | Storage in | Carbon Dioxide | Low temperature (average about 13°C.) |
| II | " over | Water | |
| III | " " | Concent. Sulphuric Acid | |
| IV | " " | Calcium Chloride | |
| V | " in | Air. | |
| VI | " " | Carbon Dioxide | Ø1.5°C in gas incubator. |
| VII | " over | Water | |
| VIII | " " | Concentrated Sulphuric Acid. | |
| IX | " " | Calcium Chloride | |
| X | " in | Air | |

The seeds for each storage treatment were placed in desiccators. The Carbon Dioxide was changed daily in the case of the two Carbon Dioxide treatments and a fresh supply of air was blown in daily in the case of the two air treatments. The concentrated

Sulphuric acid and Calcium chloride were unchanged throughout the experiment.

Each storage treatment contained 24 seeds. Not all the seeds harvested from the 80 plants were used for the randomization among the treatments, since a balance could not then have been struck for each treatment. The left-overs were therefore used as follows. Thirty of them were taken for an estimation of water content of the seeds on harvesting. These were carefully selected for pod position and seed position in the pod so that a true average figure for moisture content could be got. The remainder of the left overs were also carefully distributed through the ten treatments so that some seeds were available each time seeds out of a particular treatment were taken out for experimentation.

The technique used for moisture estimations is identical to that described in the previous chapter.

At fortnightly intervals a definite number of previously determined seeds were taken out of each of the storage treatments. The seeds were individually weighed, and their course of water intake followed according to the method described in the previous chapter.

The soaking treatment for the seeds was carried out at room temperature which was approximately constant at 17°C.

As soon as a seed had become completely hydrated

it was placed in moist sand until it had germinated and then sown in a six inch pot. The method of sowing is exactly similar to that described in the previous chapter.

Experimental Results.

(a) The moisture content of freshly harvested seeds.

The seeds which were used for moisture estimations immediately after harvesting are given in Table XIV together with the amount of water contained by each seed expressed as a percentage of its fresh weight.

If these figures are treated statistically a point emerges, which though not germane to the subject of this chapter is of sufficient interest to justify digression. A negative correlation of .701 is obtained between the figures for water content and dry matter of the seeds. In other words the smaller the seed the higher its water content per unit of dry matter. It has already been shown that the small seed absorbs more water per unit of dry weight during hydration than the large seed, and it has also been shown that the small seed has a smaller water holding capacity than the large seed when placed in the same storage conditions. It has been suggested that the greater water absorbing capacity per unit of dry weight of the small seed as compared to the large seed is due to the greater dryness of the former having in some way affected the state of the colloids of the small seed. The new finding however does not lend support to this hypothesis. Rather does it support the altemnative suggestion previously made that the small seed is an immature seed and that therefore the seed constituents are neither chemically nor physically similar to the

TABLE XIV. Moisture content of seeds immediately after harvesting and before storage.

| Seed No. | Wt. of seed before heating in grams. | Wt. of seed after heating in grams. | Loss in Wt. in grams. | Loss as % of original wt. |
|---|---|---|---|---|
| 76.1.2 | 2.564 | 1.074 | 1.490 | 58.1 |
| 38.1.5 | 2.365 | .685 | 1.680 | 71.0 |
| 3 .1.4 | 1.944 | .758 | 1.186 | 61.0 |
| 61.1.3 | 2.703 | 1.044 | 1.659 | 61.4 |
| 4 .1.1 | 1.449 | .720 | ,729 | 50.3 |
| 13.2.1 | 2.985 | 1.010 | 1.975 | 66.2 |
| 19.2.3 | 3.103 | 1.263 | 1.840 | 59.3 |
| 17.2.2 | 2.192 | .745 | 1.447 | 66.0 |
| 6 .2.6 | 2.427 | .997 | 1.430 | 58.9 |
| 3 .2.5 | .946 | .259 | .687 | 72.7 |
| 60.3.6 | 1.954 | .698 | 1.256 | 64.3 |
| 58.3.1 | 3.888 | 1.338 | 2.550 | 65.6 |
| 26.3.5 | 3.493 | 1.302 | 2.191 | 62.8 |
| 7 .3.4 | 1.924 | .871 | 1.053 | 54.7 |
| 6 .3.3 | 3.063 | 1.175 | 1.888 | 61.7 |
| 74.4.3 | 3.019 | .969 | 2.050 | 67.9 |
| 72.4.4 | .471 | .077 | .394 | 83.7 |
| 37.4.5 | 2.483 | .841 | 1.642 | 66.1 |
| 29.4.6 | 2.649 | .899 | 1.750 | 66.1 |
| 6 .4.1 | 1.636 | .511 | 1.125 | 68.7 |
| 48.5.6 | 2.290 | .786 | 1.504 | 65.7 |
| 51.5.3 | 2.286 | .591 | 1.695 | 74.2 |
| 54.5.1 | 2.210 | .656 | 1.554 | 70.3 |
| 48.5.4 | 2.334 | .837 | 1.497 | 64.1 |
| 25.5.5 | .738 | .114 | ,624 | 84.6 |
| 49.6.1 | 2.018 | .649 | 1.363 | 67.6 |
| 52.6.5 | | | | |
| 52.6.4 | 2.183 | .773 | 1.410 | 64.6 |
| 38.6.3 | .982 | .204 | .778 | 79.3 |
| 45.6.1 | 1.846 | .486 | 1.360 | 73.7 |

constituents of the big seed. It is a well-known fact that as a seed matures and develops the chemical changes which go on are accompanied by a progressive loss of water. Generally speaking therefore it is reasonable to assume that the fresh seed with a high water content is a less mature seed than that with a lower water content. We may therefore conclude that the higher water absorbing capacity per unit of dry matter of small seeds as compared to the heavier seeds is due to the relative immaturity of the small seed. This immaturity probably expresses itself as a difference in the chemical and physical structure of the colloids of the seed, having very different properties than the colloids of more mature seeds. Two of these properties would appear to be a high water absorbing capacity but a low water holding power.

(b) The effect of storage conditions on the hydration of the seed.

The results obtained for the hydration of seeds of different treatments have been condensed as far as possible but it has been found necessary nevertheless, to present the data in full so that the evidence available should be as complete as possible Tables XVa, XVb, XVc, XVd, and XVI, give the figures obtained for the Sulphuric Acid and Calcium Chloride treatments of both high and low temperature. In Table XVII the figures for weight before soaking, water absorbed, time to commencement of hydration, and corrected time to complete hydration, have been

TABLE XVA. Seeds stored over $H_2SO_4$ at low temperature.

| Seed No. | Date of soaking | Dry Weight grams. | Complete hydration weight. grams | water intake grams | hours to commencement of hydration hours. | corrected time to complete hydration = T hours. | water absorbed / dry weight = y | $\frac{y}{t}$ |
|---|---|---|---|---|---|---|---|---|
| 3.1.3 | ) | 1.730 | 3.210 | 1.480 | 0 | 27 | .855 | .032 |
| 19.1.1 | )20.10.34 | 1.815 | 3.015 | 1.200 | 0 | 18½ | .661 | .036 |
| 38.1.2 | ) | 1.241 | 1.893 | .652 | 0 | 18½ | .525 | .028 |
| 57.1.1 | ) | 1.325 | 1.902 | .577 | 0 | 8½ | .435 | .051 |
| 3.4.3 | ) | | | | | | | |
| 48.4.1 | ) 3.11.34 | .154 | Seed coat cracked during hydration. | | | | | |
| 47.4.2 | ) | .613 | 1.381 | .768 | 0 | 16 | 1.252 | .078 |
| 35.4.2 | ) | 1.191 | 2.585 | 1.394 | 3 | 22½ | 1.170 | .052 |
| | | .554 | 1.671 | 1.117 | 2 | 21 | 2.015 | .096 |
| 3.2.3 | ) | .375 | 1.174 | .799 | 0 | 28 | 2.130 | .076 |
| 6.2.1 | )17.11.34 | 1.151 | 3.085 | 1.934 | 6 | 32 | 1.680 | .052 |
| 16.2.2 | ) | 1.072 | 3.200 | 2.128 | 12 | 22 | 1.984 | .090 |
| 28.2.4 | ) | 1.109 | 2.520 | 1.411 | 13 | 25 | 1.272 | .051 |
| 69.2.1 | ) | 1.009 | 2.547 | 1.538 | 132 | 36½ | 1.524 | .042 |
| 13.5.2 | ) | .778 | 2.038 | 1.260 | 0 | 25½ | 1.619 | .063 |
| 27.5.4 | )30.11.34 | .714 | 1.946 | 1.232 | 83 | 27 | 1.725 | .064 |
| 34.5.1 | ) | .857 | Seed coat damaged owing to parasite. | | | | | |
| 59.5.2 | ) | 1.139 | 2.877 | 1.738 | 36 | 32 | 1.525 | .048 |
| 6.3.1 | ) | 1.486 | 3.666 | 2.180 | 40 | 32 | 1.467 | .046 |
| 28.3.2 | ) | .941 | 2.396 | 1.455 | 23 | 30½ | 1.545 | .050 |
| 44.3.1 | )14.12.34 | .372 | 1.198 | .826 | 0 | 26 | 2.220 | - |
| 60.3.2 | ) | .802 | 1.883 | 1.081 | 72 | 64½ | 1.348 | .021 |
| 38.6.1 | ) | .305 | .929 | .624 | 0 | 30 | 2.045 | - |

TABLE XVB. Seeds stored over $H_2SO_4$ at 31.5° C.

| Seed No. | Date of soaking | Dry weight grams. | Complete hydration weight. grams. | water intake grams. | hours to commencement of hydration. hours. | Corrected time to complete hydration hours = t. | $H_2O$ absorbed / dry weight = y | $\frac{y}{t}$ |
|---|---|---|---|---|---|---|---|---|
| 10.1.2 | | .672 | 1.909 | 1.237 | 6 | 27 | 1.840 | .068 |
| 52.1.1 | 20.10.34 | 1.130 | 2.589 | 1.459 | 17 | 16 | 1.291 | .081 |
| 61.1.2 | | 1.099 | 2.561 | 1.462 | 72 | 32½ | 1.330 | .041 |
| 29.4.2 | | .808 | 2.367 | 1.559 | 37 | 36 | 1.928 | .054 |
| 51.4.3 | 3.11.34 | .629 | 2.122 | 1.493 | 26 | 24 | 2.372 | .099 |
| 72.4.1 | | .250 | .896 | .646 | 3 | 20 | 2.582 | .129 |
| 74.4.1 | | .926 | 2.835 | 1.909 | 327 | 38 | 2.960 | .054 |
| 4.2.4 | | .687 | 1.525 | .838 | 15½ | 22½ | 1.220 | .054 |
| 7.2.2 | 17.11.34 | .888 | 1.864 | .976 | 6½ | 19½ | 1.099 | .056 |
| 20.2.1 | | 1.097 | 2.460 | 1.363 | 6½ | 27½ | 1.242 | .045 |
| 30.2.3 | | 1.623 | 3.728 | 2.105 | 72 | 13 | 1.297 | .100 |
| 61.2.4 | | 1.043 | 2.607 | 1.564 | 28½ | 33½ | 1.500 | .045 |
| 76.2.2 | | .490 | Coat cracked during hydration. | | | | | |
| 23.5.1 | | .642 | 1.971 | 1.329 | 6 | 20 | 2.069 | - |
| 29.5.3 | 30.11.34 | .667 | 1.758 | 1.091 | 12 | 14½ | 1.636 | .113 |
| 48.5.2 | | .808 | Cracked coat after 132 hours in water. | | | | | |
| 64.5.1 | | .761 | 2.218 | 1.457 | 12 | 16½ | 1.914 | .116 |
| 18.3.2 | | Cracked during storage. | | | | | | |
| 30.3.1 | | .640 | 1.695 | 1.055 | 0 | 23 | 1.648 | - |
| 47.3.2 | | .996 | Coat cracked during hydration. | | | | | |
| 67.3.1 | 14.12.34 | 1.160 | 3.006 | 1.846 | 23 | 49 | 1.590 | .027 |
| 52.6.2 | | .786 | 2.442 | 1.656 | 17 | 26½ | 2.105 | .079 |
| 68.6.1 | | .945 | Coat cracked during hydration. | | | | | |

TABLE XVC. Hydration of seeds stored over $CaCl_2$ at Low temperature.

| Seed No. | Date of soaking | Dry weight grams. | Complete hydration weight. grams. | water intake grams. | hours to commence-ment of hydration hours. | Corrected time to complete hydration hours = t. | Water absorbed / dry weight = y | y/t |
|---|---|---|---|---|---|---|---|---|
| 6.1.1 | ) | 1.098 | 1.572 | .474 | 0 | 10½ | .431 | .041 |
| 19.1.2 | ) | 1.962 | 3.013 | 1.087 | 0 | 13½ | .565 | .042 |
| 38.1.3 | )20.10.34 | 1.789 | 2.573 | .784 | 0 | 12 | .438 | .036 |
| 57.1.2 | ) | .718 | 1.056 | .338 | 0 | 13 | .471 | .036 |
| 23A.4.1 | ) | .335 | 1.433 | 1.098 | 0 | 23 | 3.245 | .141 |
| 48 .4.2 | ) | 1.077 | 2.077 | .930 | 0 | 11½ | .863 | .075 |
| 47 .4.3 | ) 3.11.34 | 1.263 | 2.691 | 1.428 | 0 | 23½ | 1.130 | .048 |
| 35 .4.1 | ) | .836 | 1.814 | .978 | 0 | 23 | 1.170 | .051 |
| 3.2.4 | ) | 1.478 | 3.483 | 2.005 | 3½ | 36½ | 1.356 | .037 |
| 6.2.2 | ) | 1.204 | 3.162 | 1.958 | 1½ | 32½ | 1.627 | .050 |
| 16.2.1 | )17.11.34 | 1.020 | 2.979 | 1.909 | 5 | 35 | 1.870 | .053 |
| 28.2.3 | ) | 1.163 | 2.540 | 1.377 | 2½ | 31½ | 1.184 | ..037 |
| 69.2.2 | ) | 1.242 | 3.011 | 1.769 | 156 | 78 | 1.424 | .018 |
| 13.5.1 | ) | .354 | 1.380 | 1.026 | 0 | 22 | 2.895 | - |
| 27.5.3 | )30.11.34 | .555 | 1.373 | .818 | 68 | 28 | 1.474 | |
| 34.5.2 | ) | .821 | Coat damaged by parasite. | | | | | |
| 59.5.1 | ) | 1.200 | 2.520 | 1.320 | 22 | 22½ | 1.085 | .0484 |
| 6.3.2 | ) | .970 | 2.371 | 1.401 | 12 | 52 | 1.445 | - |
| 28.3.1 | ) | 1.027 | 2.481 | 1.454 | 17 | 36½ | 1.416 | .039 |
| 44.3.2 | ) | .520 | 1.566 | 1.046 | 0 | 25 | 2.010 | - |
| 60.3.1 | )14.12.34 | .737 | Cracked in storage. | | | | | |
| 38.6.2 | ) | Lost. | | | | | | |
| 62.6.1 | ) | Lost. | | | | | | |

TABLE XVD. Seeds stored over $CaCl_2$ at 31.5° C.

| Seed No. | Date of soaking | Dry weight grams | Complete hydration weight grams. | Water intake grams | Hours to commencement of hydration | Corrected time to complete hydration hours. = t | Water absorbed / Dry weight = y | $\frac{y}{t}$ |
|---|---|---|---|---|---|---|---|---|
| 10.1.3 | | .270 | 1.312 | 1.042 | 0 | 27 | 3.830 | .143 |
| 24.1.1 | 20.10.34 | .429 | 1.349 | .919 | 0 | 20 | 2.140 | .107 |
| 52.1.2 | | 1.199 | 2.733 | 1.534 | 3 | 24 | 1.280 | .053 |
| 69.1.1 | | .973 | 2.235 | 1.262 | 5 | 22 | 1.296 | .059 |
| 29.4.3 | | .814 | 2.147 | 1.333 | 16 | 34 | 1.634 | .048 |
| 60.4.1 | 3.11.34 | .758 | 1.886 | 1.128 | 32 | 24 | 1.487 | .062 |
| 72.4.2 | | .126 | .496 | .370 | 2 | 13½ | 2.930 | .217 |
| 80.4.2 | | 1.050 | 3.202 | 2.152 | 22 | 28 | 2.047 | .073 |
| 5.2.1 | | 1.160 | 2.665 | 1.505 | 7 | 42½ | 1.298 | .031 |
| 7.2.3 | 17.11.34 | .768 | 1.733 | .965 | 0 | 18½ | 1.256 | .068 |
| 30.2.2 | | 1.768 | 4.144 | 2.358 | 33 | 29 | 1.320 | .045 |
| 68.2.1 | | .746 | 1.930 | 1.184 | 12 | 32 | 1.587 | .049 |
| 76.2.3 | | .457 | 1.548 | 1.091 | 26 | 41 | 2.385 | .058 |
| 25.5.4 | | 1.133 | 2.719 | 1.586 | 12 | 31½ | 1.400 | .044 |
| 29.5.2 | 30.11.34 | .211 | .830 | .619 | 5 | 22 | 2.933 | - |
| 40.5.1 | | .844 | 2.550 | 1.706 | 68 | 42 | 2.022 | .048 |
| 80.5.2 | | Lost. | | | | | | |
| 20.3.1 | | 1.145 | Cracked during hydration. | | | | | |
| 33.3.2 | | .718 | 1.994 | 1.276 | 0 | 26 | 1.776 | - |
| 48.3.1 | 14.12.34 | .789 | 1.905 | 1.116 | 16 | 28 | 1.414 | - |
| 70.3.2 | | Cracked in storage. | | | | | | |
| 58.6.1 | | 1.005 | 2.768 | 1.763 | 10 | 22 | 1.754 | - |
| 70.6.2 | | .941 | Cracked during hydration. | | | | | |

TABLE XVI. Moisture estimations - Seeds over $CaCl_2$ and $H_2SO_4$

October 20th 1934

| Seed No. | Treatment | Weight of seed before heat. grs. | Weight after heat. grs. | Loss in weight. grs. | Loss as % Of dry matter. | Average % loss. |
|---|---|---|---|---|---|---|
| 48.11.3 | )$H_2SO_4$ | .273 | .124 | .149 | 54.5 | ) |
| 76. 1.3 | )low | 1.083 | .871 | .212 | 19.8 | ) 31.7 |
| 64. 2.1 | )temp. | .984 | .779 | .205 | 20.8 | ) |
| 48.11.2 | )$CaCl_2$ | .583 | .459 | .124 | 21.3 | ) |
| 62. 3.1 | )low | .309 | .051 | .258 | 83.5 | ) 52.1 |
| 57. 1.3 | )temp. | .678 | .327 | .351 | 51.7 | ) |
| 54.10.1 | )$H_2SO_4$ | .535 | .498 | .036 | 6.7 | ) |
| 47. 3.3 | )high | .985 | .912 | .073 | 7.4 | ) $\frac{21.4}{3}$ = 7.1 |
| 24. 2.2 | )temp. | .288 | .267 | .021 | 7.3 | ) |
| 58. 9.2 | )$CaCl_2$ | .746 | .671 | .075 | 10.5 | ) |
| 71. 5.3 | )high | .181 | .160 | .021 | 11.6 | ) $\frac{33.4}{3}$ = 11.1 |
| 2. 2.2 | )temp. | .962 | .849 | .113 | 11.7 | ) |
| November 3rd, 1934. | | | | | | |
| 79.10.1 | )$H_2SO_4$ | .371 | .312 | .059 | 15.9 | ) |
| 70. 3.5 | )low | .618 | .515 | .103 | 16.6 | ) $\frac{45.8}{3}$ = 15.3 |
| 78. 4.1 | )tempera. | .667 | .578 | .089 | 13.3 | ) |
| 79. 9.1 | )$CaCl_2$ | .388 | .292 | .096 | 24.7 | ) |
| 48. 3.3 | )low | .990 | .742 | .248 | 25.0 | ) $\frac{76.4}{3}$ = 25.5 |
| 29. 4.5 | )temp. | 1.043 | .765 | .278 | 26.7 | ) |
| 62. 9.1 | )$H_2SO_4$ | .579 | .544 | .035 | 6.0 | ) |
| 20. 3.3 | )high | 1.188 | 1.118 | .070 | 5.9 | ) $\frac{19.8}{3}$ = 6.6 |
| 44. 3.3 | )temp. | .202 | .186 | .016 | 7.9 | ) |
| 30. 9.1 | )$CaCl_2$ | .774 | .720 | .054 | 7.0 | ) |
| 28. 8.6 | )high | .104 | .095 | .009 | 8.7 | ) $\frac{24.1}{3}$ = 8.0 |
| 63.4.1 | )temp. | .287 | .263 | .024 | 8.4 | ) |

TABLE XVI (contd).

November 17th 1934.

| Seed No. | Treatment | Weight of seed before heat. grs. | Weight after heat. grs. | Loss in weight grs. | Loss as % of dry matter | Average % loss |
|---|---|---|---|---|---|---|
| 55.14.1 | $H_2SO_4$ | .211 | .186 | .025 | 11.8 | |
| 48. 5.5 | low | .995 | .895 | .100 | 10.5 | $\frac{33.7}{3} = 11.2$ |
| 24.1 .4 | temp. | .411 | .364 | .047 | 11.4 | |
| 34.16.1 | $CaCl_2$ | .491 | .440 | .051 | 10.4 | |
| 70. 4.4 | low temp. | .310 | .275 | .035 | 11.3 | $\frac{35.4}{3} = 11.8$ |
| 12. 1.1 | | .626 | .540 | .086 | 13.7 | |
| 46.8.3 | $H_2SO_4$ | 1.064 | 1.011 | .053 | 5.0 | |
| 30.2.6 | high | 1.601 | 1.523 | .078 | 4.9 | $\frac{14.8}{3} = 4.9$ |
| 67.5.1 | temp. | .307 | .292 | .015 | 4.9 | |
| 40.8.2 | $CaCl_2$ | .417 | .391 | .026 | 6.2 | |
| 6 .2.5 | high | 1.221 | 1.154 | .067 | 5.5 | $\frac{15.2}{3} = 5.1$ |
| 34.8.1 | temp | .114 | .110 | .004 | 3.5 | |
| November 30th 1934. | | | | | | |
| 28.2.5 | $H_2SO4$ | .833 | .749 | .084 | 10.1 | |
| 46.9.3 | low | 1.425 | 1.290 | .135 | 9.5 | $\frac{31.3}{3} = 10.4$ |
| 70.6.3 | temp. | 1.001 | .884 | .117 | 11.7 | |
| 31.2.1 | $CaCl_2$ | .303 | .269 | .034 | 11.2 | |
| 52.7.3 | low | .158 | .142 | .016 | 10.1 | $\frac{31.3}{3} = 10.4$ |
| 34.8.2 | temp. | .726 | .655 | .071 | 9.8 | |
| 64.5.3 | $H_2SO4$. | .713 | .677 | .036 | 5.0 | |
| 67.4.1 | high | .184 | .173 | .011 | 6.0 | $\frac{17.4}{3} = 5.8$ |
| 63.3.1 | temp. | .388 | .363 | .025 | 6.4 | |
| 34.7.1 | $CaCl_2$ | .576 | .540 | .036 | 6.3 | |
| 33.3.3 | high | .792 | .746 | .046 | 5.8 | $\frac{17.6}{3} = 5.9$ |
| 60.3.4 | temp. | .798 | .754 | .044 | 5.5 | |

TABLE XVII. Compound of Tables XVa, XVb, XVc, XVd, and XVI.

| Date of soaking | Wt. of seed in grams. | Water taken in in grams | Water content of seeds. % of seed Wt. | $\frac{H_2O \text{ intake}}{\text{Dry Wt.}} = y$ | Hours to commencement of hydration. | Correct time to compl. hyd. = t hours | $\frac{y}{t}$ |
|---|---|---|---|---|---|---|---|
| 20.10.34 | | | | | | | |
| $H_2SO_4$ low temp. | 1.528 | .977 | 31.7 | .639 | 0 | 18 | .035 |
| $H_2SO_4$ high temp. | .967 | 1.386 | 7.1 | 1.433 | 32 | 25 | .057 |
| $CaCl_2$ low temp. | 1.383 | .671 | 52.1 | .485 | 0 | 12 | .040 |
| $CaCl_2$ high temp. | .718 | 1.189 | 11.1 | 1.656 | 2 | 23 | .072 |
| 3.11.34 | | | | | | | |
| $H_2SO_4$ low temp. | .786 | 1.093 | 15.3 | 1.390 | 2 | 20 | .069 |
| $H_2SO_4$ high temp | .653 | 1.402 | 6.6 | 2.145 | 98 | 29 | .074 |
| $CaCl_2$ low temp. | .878 | 1.108 | 25.5 | 1.261 | 0 | 20 | .063 |
| $CaCl_2$ high temp. | .687 | 1.246 | 8.0 | 1.814 | 18 | 25 | .073 |
| 17.11.34 | | | | | | | |
| $H_2SO_4$ low temp. | .927 | 1.568 | 11.2 | 1.690 | 8 | 27 | .062 |
| $H_2SO_4$ high temp. | 1.074 | 1.295 | 4.9 | 1.205 | 25 | 21 | .057 |
| $CaCl_2$ low temp | 1.216 | 1.812 | 11.8 | 1.488 | 3 | 34 | .044 |
| $CaCl_2$ high temp. | 1.238 | 1.609 | 5.1 | 1.298 | 13 | 30 | .043 |

TABLE XVII. (contd.)

| Date of soaking | Wt. of seed in grams. | Water taken in in grams | Water content of seeds. % of seed Wt. | $\frac{H_2O \text{ intake}}{\text{Dry Wt.}} = y$ | Hours to commencement of hydration. | Correct time to compl. hyd. = t hours. | $\frac{y}{t}$ |
|---|---|---|---|---|---|---|---|
| 30.11.34 | | | | | | | |
| $H_2SO_4$ low temp. | .910 | 1.442 | 10.4 | 1.584 | 63 | 30 | .053 |
| $H_2SO_4$ high temp. | .778 | 1.360 | 5.8 | 1.747 | 14½ | 22 | .080 |
| $CaCl_2$ low temp. | .838 | 1.233 | 10.4 | 1.471 | 62 | 38 | .039 |
| $CaCl_2$ high temp. | .795 | 1.142 | 5.9 | 1.436 | 25 | 36 | .040 |
| 14.12.34 | | | | | | | |
| $H_2SO_4$ low temp. | .821 | 1.233 | 1 - | 1.501 | 27 | 36 | .042 |
| $H_2SO_4$ high temp | .862 | 1.519 | - | 1.760 | 13 | 33 | .053 |
| $CaCl_2$ low temp. | .839 | 1.300 | - | 1.549 | 15 | 38 | .041 |
| $CaCl_2$ high temp. | .837 | 1.385 | - | 1.655 | 9 | 25 | .066 |

averaged for each of the storage classes at each experimental period (date of soaking) and from these average figures the figures for "y" and y/t are obtained. "y" is the amount of water absorbed per unit of dry weight and y/t is the amount of water absorbed per unit of dry weight per unit of time.

The two sets of seeds stored over water were designed to act as controls to the Sulphuric Acid and Calcium Chloride storage classes. In spite of precautions taken the seeds stored over water became very heavily infected by a mould which was identified as one of the Penicillium species. In view of this these two storage classes were discarded early on in the experiment.

In spite of the care taken in randomization Tables XVa, XVb, XVc, and XVd show that there is a marked variation in the weight of the experimental seeds. This is understandable in view of the fact that the variation between seeds of different pods is greater than the variation of seeds within the pod as was shown in the last chapter. It would not have been correct however to place all the seeds of one plant for instance in one storage treatment and all the seeds of another plant in another treatment since it has been shown that a greater degree of variation exists between seeds of one plant and seeds of another plant than between seeds of the same plant. If the seeds of one plant were

more mature than the seeds of another plant, there would exist a constant error in the comparisons between one storage treatment and another. The randomization method adopted, though obviously not perfect, is claimed to be better than the alternative. From what is already known regarding the reaction of the seed as related to its size, we know that the differences in the weight of the seeds which still exist even after adjustment for water content, will be responsible for some differences in the hydration of the seeds. This source of error has been overcome to some extent by taking the average figures for the seeds of each storage class at each experimental period. Examination of Table XVII will show that the variation in the average figures for dry seed weight is much less than if the individual figures for dry seed weight of Tables XV a, b, c, and d, are considered.

IN Table XVI are given the moisture contents of seeds of each of the four storage classes for each of the experimental dates. The moisture content of the seeds of any one storage class at any one of the dates of soaking is represented by the average moisture content of three seeds. The figures for moisture content are included in Table XVII.

Examination of the moisture content figures in Table XVII shows that at any one period of experimentation the seeds which had been stored in Sulphuric acid at high temperature contain less

moisture than the seeds of any other storage class. This conforms to the generally accepted fact that Sulphuric acid is a stronger dehydrant than Calcium chloride. Further it is clear from the figures that in either case a high temperature favours dehydration of seeds more than a low temperature. While on the subject of moisture contents attention must be given to a phenomenon which would appear to be significant. The moisture content of the seeds over concentrated Sulphuric acid at high temperature on the 17th of November was 4.9% of the seed weight, and that on the 30th of November was 5.8%. A similar rise though not so great is shown by seeds stored over Calcium cholride at high temperature. It might be argued that this rise in moisture content is purely accidental and due to some differences in the two batches of seed on which moisture estimations were made. If this were the case the existence of the same phenomenon in both Calcium Chloride and Sulphuric acid taking place at the same time would have to be regarded as a coincidence. This is highly improbable since there is nothing in the figures of the seeds used for the moisture estimations (c.f. Table XVI) which would suggest that the rise was due to some differences in the batches of three seeds each. The only difference that could be put forward is that in the case of Sulphuric acid storage the seeds used for the moisture estimation on the 30th of November were smaller in

weight than the seeds used for the moisture estimation on the 17th of November. But it has already been shown that the small seed has a smaller water holding capacity than a big seed. In this case we find the smaller seed holding more water than the big seed even though the dehydrating action has been going on for a fortnight longer. In view of this, the rise in moisture content of seeds stored over Sulphuric Acid and Calcium Chloride at high temperature after six weeks storage, must be considered significant. Whether this rise continues as storage proceeds is not known since moisture content figures are not available for the last experimental period. This rise in moisture content is not apparent in either the seeds stored over Sulphuric acid at low temperature or Calcium chloride at low temperature.

A feature of slightly greater complexity is found in the water intake per unit of dry weight ("y") figures. Examination of the "y" figures given in Table XVII will show that in the case of seeds stored over Sulphuric acid at high temperature the amount of water absorbed per unit of dry weight increases with time of storage reaching a maximum in the fourth week. A fortnight later there is a marked drop in the same figure followed by a rise a fortnight later still (i.e. eight weeks after the commencement of storage). This increase is maintained in the tenth week.

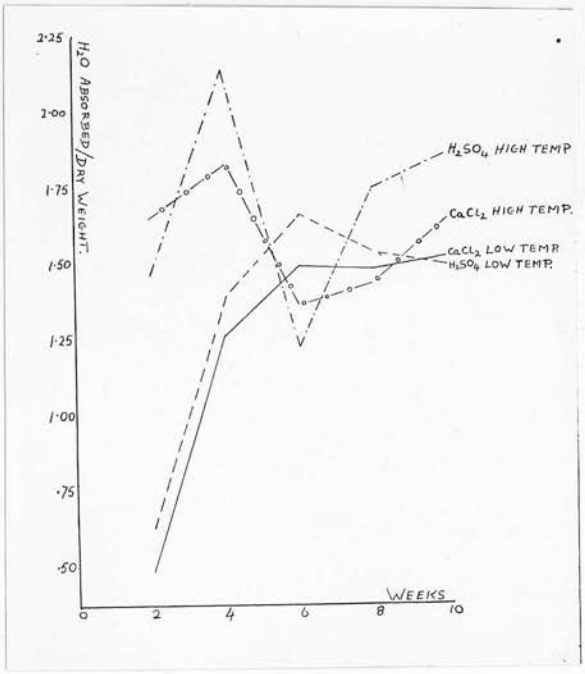


Fig. XXI.

Changes in time of the amount of water absorbed per unit of dry weight by seeds subjected to four different storage treatments.

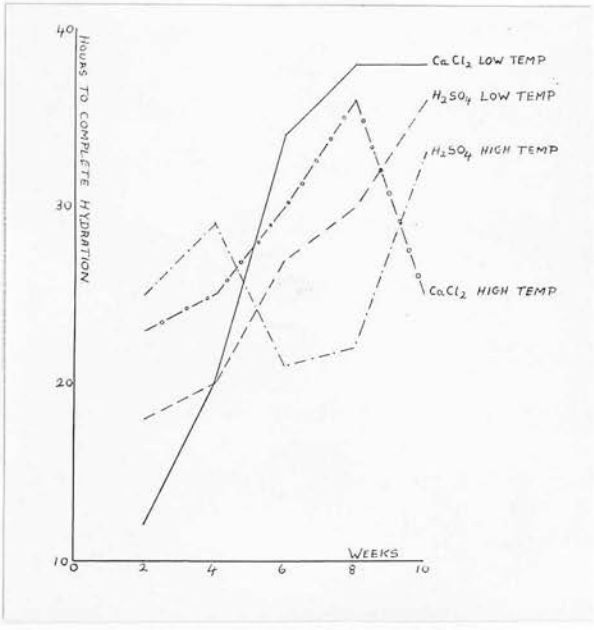


Fig. XXII.

Changes in time occupied in hydration of seeds subjected to four different storage treatments.

Exactly the same trend is shown by the "y" figures for the seeds stored over Calcium Chloride at high temperature, The seeds stored over Sulphuric acid at low temperature show a rise to a maximum after six weeks storage and thereafter a fall but no second rise as in the case of the two high temperature storage treatments. The seeds stored over Calcium chloride at low temperature show a steady rise all the way through in the amount of water absorbed per unit of dry weight, except for a slight check at the eighth week. These features are graphically represented in Fig. XXI.

In Table XVII a column of figures entitled "corrected time to complete hydration" is given. It will be remembered that this figure was used in the last chapter and was defined as the time actually taken for the seed to hydrate. The figures given in Table XVII are averages as has already been explained earlier in this section. These figures show that in the case of seeds stored over Sulphuric Acid and Calcium Chloride at low temperature the time taken for hydration increases gradually with increase in time of storage. Seeds stored over Sulphuric acid at high temperature differ in that a fall follows an initial increase in time of hydration and the fall is followed by a second rise. The Calcium chloride treatment at high temperature appears to have the same effect except that there is no second rise. These features in the hydration time

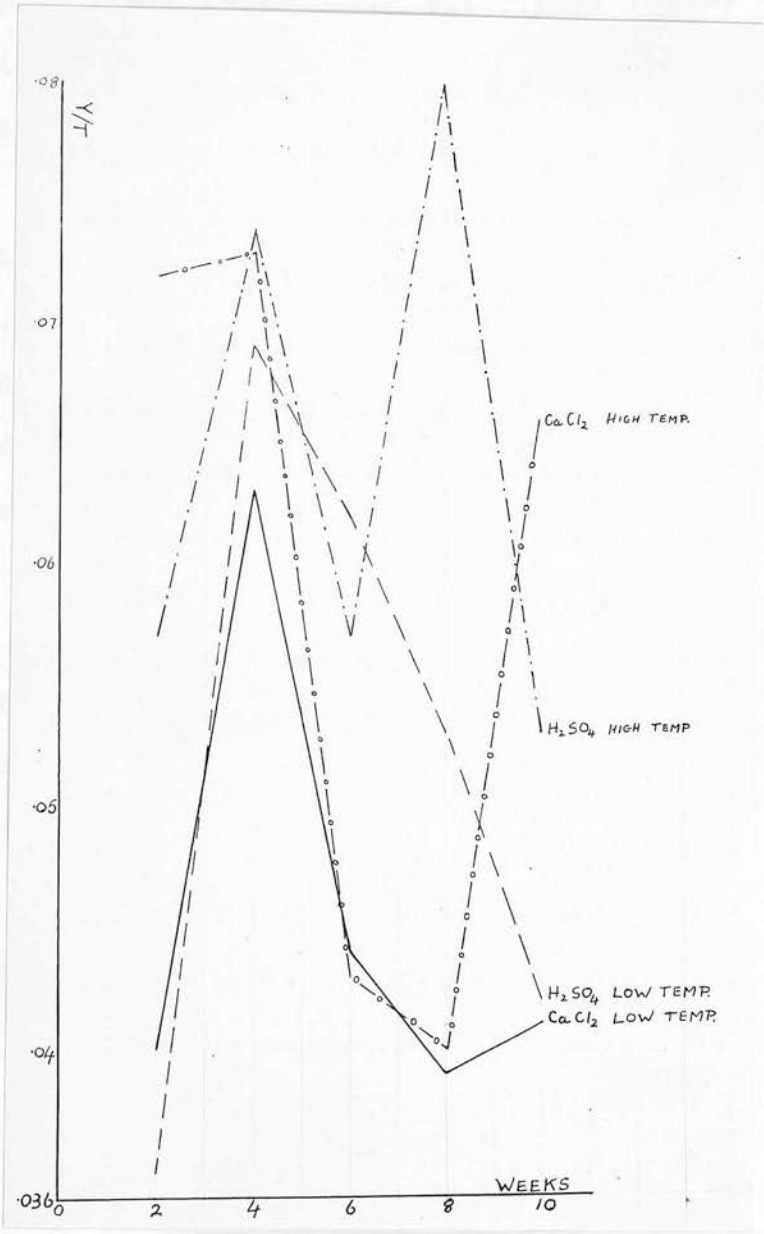


Fig. XXIII.

Changes in time of the amount of water absorbed per unit of dry weight per unit of time by seeds subjected to four different storage treatments.

figures are clearly seen in the graphs of Fig. XXll

The "y" and the corrected time to complete hydration figures are both embodied in the y/t figures of Table XVll. The y/t figure (water intake per unit of dry weight per unit of time) may therefore be called the efficiency index figure of the seeds. The two high temperature classes show violent fluctuations in their y/t figures (c.f. Fig. XXlll). There is however one essential difference between the Sulphuric acid class and the Calcium chloride class at high temperature, and that is that whereas the former class shows two maxima with a very definite drop after the second maximum, the latter class has no drop after the second maximum. There would seem to be a lag in time of the Calcium chloride high temperature class behind the Sulphuric Acid high temperature class. The form of the graphs of the two low temperature classes are essentially similar. They reach a maximum efficiency after a month of storage and thereafter gradually decrease. These two graphs differ from that of the Calcium chloride high temperature class in that they do not show a second rise.

There would therefore appear to be a graduation in the intensity of effect in given time of these four storage treatments on seeds. Storage over Sulphuric Acid at high temperature seems to have the greatest effect followed by Calcium Chloride at high temperature, while the two low temperature treatments have least effect.

The figures above discussed present a great deal of food for thought. The validity of the figures might be questioned since it is known that there is a great deal of inherent variation in the material used. It might be argued that the phenomena indicated are no more than the natural variation which exists between one sample of seed and another. This is at once admitted, but the fact that the course of the changes for each of the storage classes is uniform for each of the hydration figures considered, and further the essential similarity of these figures in the two high temperature and the two low temperature classes, leads to the conclusion that the phenomena are due to the treatment of the seed and not to variation inherent in the seed.

In the discussion of these provisional results it must be borne in mind that this field of seed investigation has been untouched so far as is known to the present writer. There is therefore no background into which the thesis here presented can be fitted, and the suggestions made as to the possible causes of the phenomena above indicated are made with the help of facts gained, from similar investigations on physically homogeneous colloids.

A fact brought out early in this section was that the four storage treatments were very definitely graded in their dehydrating efficiency, The order from greatest to lowest efficiency was:-

I Sulphuric Acid high temperature.
II Calcium Chloride high temperature.
III Sulphuric Acid low temperature.

IV Calcium Chloride low temperature.

Further it has been pointed out that the magnitude of the effect produced on the seed as measured by the amount of change in hydration characteristics, also varies from one storage treatment to the other. The order from greatest to least effect is exactly the same as that given for dehydrating efficiency. It would be a reasonable inference therefore, that the dehydration efficiency of the storage treatment is responsible for the amount of change in given time in hydration behaviour of the seed. There are good grounds for doubting however whether the matter is as simple as this. The moisture contents of seeds stored over Calcium Chloride and Sulphuric Acid at high temperature do not show a gradual fall in time to a constant figure as would be expected, but after an initial gradual fall a rise takes place. This would indicate that either the moisture holding capacity of the seed had changed or that the dehydrant had become suddenly less efficient thus altering the equilibrium between seed and dehydrant and that the equilibrium was again restored by a slight hydration of the seed. There is no reason to believe that the dehydrant had in fact become less powerful. It is suggested therefore that the more probable explanation of the rise in moisture content of seeds stored at high temperature is that the moisture holding capacity of the seed had changed in other words some fundamental change had taken

place in the substance of the seed. This change might well be a change in the structure of the colloid. The drying efficiency of Sulphuric Acid and Calcium-Chloride at low temperature is not as great as that at high temperature and the reason for the non-existence of the rise in the moisture content of the seeds in these storage classes is that the critical point, at which further dehydration of the colloid causes a change in structure, is never reached in the storage time of these experiments.

To account for the changes in time of any of the hydration figures discussed, however, it is necessary that we postulate other critical points in the history of the seed during the storage period. Fig. XXI shows for example that in the case of seeds stored in either of the two high temperature classes, the amount of water absorbed per unit of dry weight rises, falls and rises again as time in storage proceeds. It is suggested that during the storage period three changes took place in the seed each of which had the effect of changing the trend of the amount of water absorbed per unit of dry weight.

Whatever the explanation of the phenomena above indicated it would seem that hysteresis of the seed colloids plays an important part in determining the hydration behaviour of the seeds. Manifestly this aspect is of the utmost importance for future studies.

The time actually taken for the seed to hydrate

has been considered in this section in relation to the storage treatment of the seed, but nothing has yet been said regarding the effect of storage upon the initial soaking period during which no increase in weight of the seed takes place. This "time to commencement of hydration" was defined and discussed in the previous chapter.

The average figures for this period are given in Table XVII for each storage class at each experimental period. It will be noted that this period is non-existent a fortnight after commencement of storage, in the two low temperature classes, while it is very short if present at all a month after storage in the same two classes. Examination of the moisture content figures corresponding to the "time to commencement of hydration" figures, will show that the moisture content of the seeds has to be below 20% of the weight of the seed before the period between beginning of soaking and commencement of hydration appears.

The course of the changes in "time to commencement of hydration" for each storage class are shown graphically in Fig. XXIV. These graphs show that with the exception of the Calcium Chloride high temperature class, the course of changes are similar. There is however one important difference; while the three classes all show a rise to a maximum time to commencement of hydration followed later by a decrease in this interval, the maximum is reached much earlier in the case of the Sulphuric Acid high temperature class

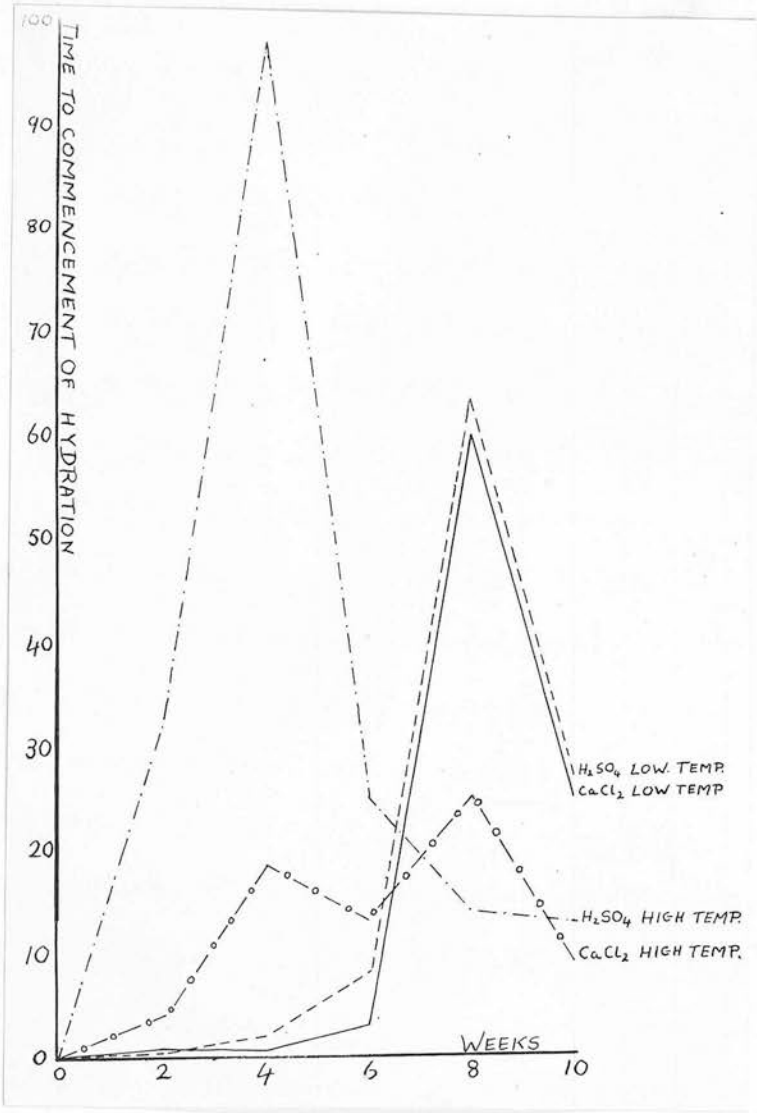


Fig. XXIV.

Changes in time of the time taken to commence hydration by seeds subjected to four different storage treatments.

than in the case of the two low temperature classes. This again indicates the lag in time of the effect of the two low temperature classes behind the two high temperature classes. But what is the reason for the decrease in the interval to commencement of hydration after a maximum is reached? It is to be found in Tables XV a,b,c, and d. Examination of these Tables show that a number of the seeds of the high temperature classes showed cracks in their coats, either before or after soaking began, towards the end of the storage period. These seeds were of course discarded but the possibility remains that the seeds which appeared normal and with no cracks may have had in fact small cracks which could not be detected. These small cracks would act as channels for water thus quickening the onset of hydration. The largest proportion of cracked seeds ocurred in the Sulphuric Acid high temperature, while none ocurred in the low temperature classes. It is suggested that the cracking of the seed coat is due to the stresses set up in the seed coat as a result of strong dehydration.

The Carbon Dioxide and Air Storage treatments produced no results of outstanding significance. The seeds of the Air treatments suffered the same fate as did the seeds of the Water treatments; shortly after storage began they were all attacked by Penicillium and had to be discarded. The seeds in the two Carbon Dioxide treatments changed gradually

in colour from the light green of the freshly harvested seed to a uniform chocolate brown, the process being more rapid at high temperature than at low temperature. The explanation may possibly be that the browning is a result of the action of the products of anaerobic respiration on the seed coat.

(c) The effect of seed storage on the growth of the resulting plant.

On Table XVIII are given the details in brief of the seeds which germinated and produced plants and the seeds which did not germinate of each of the Sulphuric Acid and Calcium Chloride treatments. Examination of this table will show that, of the plants now growing, eight originated from seeds stored over Calcium chloride at low temperature, four from Sulphuric acid at low temperature, one from Calcium chloride at high temperature and none from Sulphuric acid at high temperature. This order is exactly the reverse of that indicated in the previous section for dehydration state of the tissues of the seeds under each of the storage conditions. This latter order was also the order of greatest to least effect on the colloids of the seeds. It would appear therefore that a storage treatment which has the greatest effect on the colloids of the seed is least suitable for the production of good seed, and from this we come to the conclusion that hysteresis of the seed colloids has a marked effect on the germination of the seed and the plant it produces.

TABLE XVIII. Fate of plants produced by seeds stored over $CaCl_2$ and $H_2SO_4$ at low and high temperature.

| Date of soaking | Plants growing | Seed decayed before germination. | Germinated but plumule did not appear. | Died after appear of plumule | Seeds discarded before sowing. |
|---|---|---|---|---|---|
| Seeds stored over $CaCl_2$ at low temperature. | | | | | |
| 20.10.34 | 3 | | | 1 | |
| 3.11.34 | | 4 | | | |
| 17.11.34 | 1 | 2 | | | |
| 30.11.34 | 3 | 1 | | | 1 |
| 14.12.34 | 1 | 1 | 1 | | 3 |
| Seeds stored over $CaCl_2$ at high temperature. | | | | | |
| 20.10.34 | 1 | 1 | | 2 | |
| 3.11.34 | | 4 | | | |
| 17.11.34 | | 2 | 1 | | |
| 30.11.34 | | 5 | | | 1 |
| 14.12.34 | | 3 | | | 3 |
| Seeds stored over $H_2SO_4$ at low temperature. | | | | | |
| 20.10.34 | 2 | 1 | | 1 | |
| 3.11.34 | | 3 | | | 1 |
| 17.11.34 | | 2 | | 2 | |
| 30.11.34 | 1 | 2 | | 1 | 1 |
| 14.12.34 | 1 | 4 | | | |
| Seeds stored over $H_2SO_4$ at high temperature. | | | | | |
| 20.10.34 | | 1 | | 2 | |
| 3.11.34 | | 4 | | | |
| 17.11.34 | | 1 | 3 | | |
| 30.11.34 | | 4 | | | 2 |
| 14.12.34 | | 3 | | | 3 |

This conclusion is further supported by consideration of the number of seeds which produce seedlings and the fate of these seedlings of the two low temperature classes. There were ten seedlings from the Calcium chloride low temperature class and eight from the Sulphuric acid low temperature class. This in itself is an insignificant difference, but only two seedling later died from the Calcium chloride class while four of the Sulphuric acid class died. The majority of deaths in seedlings was due to attack by Thielavia species. The figures suggest the greater vigour of seedlings produced from seeds stored over Calcium chloride at low temperature, and this greater vigour is shown in the greater resistance to parasitic invasion. It will be remembered that storage of seeds over Calcium chloride at low temperature is not so drastic as measured by the effect on the colloid as storage over Sulphuric Acid at low temperature. The difference in effect on the colloid is admittedly small, but even this small difference it is submitted could account for the difference in the vigour of the seedlings produced from seeds of the two storage classes.

One or two of the Carbon Dioxide seeds stored at low temperature germinated when placed in moist sand after a fortnight in storage, but otherwise seeds stored in Carbon Dioxide either at high or low temperature failed to germinate and decayed rapidly.

## Summary of Conclusions.

I A negative correlation of ,701 is obtained between the water content and dry matter of freshly harvested seeds. This is regarded as good evidence for calling the small seed an immature seed.

II Evidence is presented to show that hysteresis of the seed colloids plays an important part in determining the hydration behaviour of the seeds.

III The initial soaking period during which no increase in weight of the seed takes place is non-existent so long as the moisture content of the seeds is above 20% of the weight of the seed. With decreasing moisture content the period lengthens to a maximum thereafter shortening rapidly as dehydration proceeds. It is shown that the decrease in "time to commencement of hydration" is probably due to the presence of small cracks in the seed coat which could not be detected.

IV The history of the seed is shown to have a predetermining influence on its germination and on the plant produced.

# Chapter VI.

## DISCUSSION.

# DISCUSSION

The researches described in the previous chapters though diverse in nature have all had as their aim the elucidation of the problem defined in the general introduction to the thesis. The problem as there outlined consisted of two main parts. The first part entailed the determination of the mechanism of water intake by the seeds of *Vicia Faba*, and the second part concerned the factors which affect that mechanism.

The conclusions reached as a result of the experiments described are sufficiently indicative to permit the formulation of a hypothesis. At the same time this would conveniently bring together into one picture the main points made in the thesis.

The mental picture of the early stages of germination conveyed by the work here reported may be briefly described as follows. Within the first half-hour of soaking, the seed absorbed a small amount of water as indicated by the slight increase in weight. It was shown by Nelson and Macsween (1933) that within 90 seconds of the seed coming into contact with water the hilar slit closes. This observation has been confirmed and must be due as these workers suggest to "colloidal swelling of one layer working over another layer which does not swell." There can be no doubt that part of the small increase in the first half-hour of soaking is due to the hydration of those palisade cells on either side of the hilar slit, but there can also be no doubt of the presence of capillary water on the seed coat which is not

removed by the drying methods used before weighing. The existence of this water is clearly demonstrated by the rapid turn of colour of anhydrous Copper Sulphate when sprinkled on the surface of a "dried" seed coat. Following this small initial intake of water which rarely exceeds .005 grs. there is usually a period of inactivity during which no water accrues to the seed. This period has been shown to vary considerably from seed to seed. The evidence clearly is that it does not exist at all if the seed has a moisture content of more than 20%, but with decreasing moisture content the length of the period increases to a maximum and then again decreases. Sooner or later following on the phase of capillary binding, a second phase sets in during which the seed increases in weight very gradually. This second phase coincides with the appearance of wrinkles in a localized area of the testa. The phenomenon of localized absorption of water by the testa as shown by the folding of the latter in restricted areas, is one which requires a good deal of further investigation. The suggestion made that the seeds derived from any one pod have their areas of localized absorption in one of two loci; is interesting in that it suggests a mechanism common to all seeds in a pod but not common to all pods on a plant. This narrows the field of future enquiry and brings out the question suggested by Nelson (1926) that the relationship of seed to containing pod is

important in determining the intake of water by the seed in the future. The localized swelling area increases in size though not necessarily equally in all directions. For instance a seed may begin to wrinkle on its non-hilar periphery but rarely does the wrinkled area extend on either side of the seed; it usually extends along the periphery. The wrinkling process which is accompanied by a progressive increase in weight of the seed, proceeds until the testa in the region of the micropyle becomes hydrated. Up to this point the micropylar canal extending from the exterior to the pocket which contains the radicle of the embryo, has been closed owing to the walls of the canal being closely pressed together. As soon as the tissues surrounding the canal become hydrated the walls separate, and with the opening of the micropylar canal there is initiated a phase in hydration of greatly increased rate of water intake. The large body of water which now enters the seed does so through the micropyle as a result of the elastic pressure developed in the testa tending to smoothen out the latter. During the wrinkling process the increase in volume of the seed is far from proportional to the amount of water entering the seed. If it is assumed that prior to hydration the pressure of the internal atmosphere of the seed was atmospheric, the increase in volume during wrinkling must be accompanied by a marked reduction of pressure within the seed. Though not

previously suggested it is quite possible that the suction force thus developed also helps to draw water through the micropyle. As more and more water is drawn through the micropyle the folds in the testa gradually disappear. Up to the time of opening of the micropyle the only portions of the cotyledons which appear to have hydrated, as indicated by the change in colour, are those portions immediately under the troughs of the wrinkled testa. In contact with free liquid the cotyledons hydrate and expand rapidly. Thus micropylar opening presents free water to the cotyledons which then behave in just the same way as when dissected out and presented with water in vitro. The phase of large increases in hourly weight of the whole seed gradually merges into a final phase of water absorption, characterised by increments of weight each of progressively decreasing amount until the seed becomes fully hydrated.

The above hypothesis is based on the results of researches described in the four preceeding chapters, and in the view of the writer is the only one which would account for the phenomena observed. Some of the hypotheses previously put forward by other workers are in direct conflict with that offered here, while others differ only in detail. It becomes necessary therefore to discuss the results and hypotheses of these workers in the light of the conclusions reached in this thesis.

Mention must first be made of the work of Nelson

and Macsween (1933) since the work here reported is concerned with the same material as these workers employed. They summarize their findings in the following terms: "The mechanism of water intake by the seed of Broad Bean is not simple but involves at least two mechanisms. In point of time the first is hydration of the colloids of the testa; this is followed by an osmotic intake of water through the semipermeable membrane of the testa, the osmotically active substance being a reducing sugar formed from carbohydrate not in the embryo itself. The passage of the water through apertures such as the micropyle or hilar slit is not significant if it takes place at all." It is apparent that the hypothesis as expressed in the above passage is basically quite different from that formulated as a result of the work here reported. According to the latter water intake by seeds is the result of only one mechanism and that is colloid hydration. Nelson and Macsween consider that colloid imbibition holds during the wrinkling period. When the wrinkles begin to disappear and free liquid appears between the testa and cotyledons so the second osmotic mechanism begins to operate. The appearance of water under the testa and the straightening out of the folds of the testa is shown to be a consequence of the opening of the micropyle canal. In spite of the presence of osmotically active Tannins and possibly Gallic acid, though certainly not reducing sugar, the writer cannot

conceive of water being drawn through the hydrated testa, which admittedly is a semipermeable membrane up to a point, when there is a pore which offers no resistance to the passage of water. The tannins and associated substances found in the testa are probably the products of metabolic changes during the ripening processes of the seed.

Pringsheim (1930) agrees with Nelson and Macsween (1933) in so far as he states that "Im ganzen hat man nicht den Eindruck, dass in der Hilarregion ein spezifisch wasseraufnehmendes Organ liegt." Further he states "Fur die Mikropyle ist diese Funktion bestimmt nicht anzunehmen." He disagrees however in stating that the mechanism which draws water into the seed is purely one of colloid hydration. Using Lupinus albus and Pisum sativum as experimental material, Pringsheim describes the curves he obtained for the increase in weight of seeds as those characteristic of physically homogenous swelling bodies. He weighed individual seeds at 1,2,3,4 and 5 hours after the commencement of soaking and found in all cases that the initial increase in weight of the seed was the greatest increase, subsequent increases becoming gradually smaller until the curve eventually became flat. In the course of general description of the external appearance of the seed during hydration he says "Bei der Quellung des Samens der Lupine wie auch anderer Leguminosen fallt die langst bekannte Tatsache auf, dass die Samenschale

schon nach einer halben Stunde Falten zeigt, welche im Verlauf der nachsten Stunden wieder verschwinden." In fact his first weighing an hour after the commencement of soaking was taken when the dewrinkling process had already set in and had been progressing for about half an hour. The results of the experiments described in Chapter III show that the dewrinkling process follows the opening of the micropylar canal through which a large body of water is drawn into the seed. The curves there given of the weight increase of individual seeds show that after the opening of the micropyle the seed increases in weight very rapidly, and indeed the curve of this second phase of the hydrating seed of Vicia Faba bears a very marked resemblance to the curve which Pringsheim gives as representing the increas in weight of a seed of Lupinus albus, from the air dry condition to complete hydration. These considerations lead us to the conclusion therefore that the divergence between the results given in this thesis and those given by Pringsheim is due to the latter's failure to appreciate the significance of the course of weight increase of the seed during the hydration stage immediately prior to the onset of dewrinkling. There is no doubt that the divergent results have been directly responsible for the differences in the conclusions reached.

Figures given by Nobbe (1876) for the weight increase of Broad Bean seeds ("Puffbohne") confirm

the results given in chapter III. He shows that initially the increase in weight of the seed is very gradual which later increases considerably and finally decreases again. This worker however makes no reference to the mechanism causing these changes. Detmer (1880) working with peas comes to the conclusion that "die Wasseraufnahme ..... zunachst nur eine geringfugige ist, dann energischer wird, um schliesslich wieder abzunehmen," which is exactly the conclusion arrived at by Nobbe and the present writer. Detmer (1898) also supports the contention put forward that the Micropyle plays an important part in the absorption of water by seeds, though he does not indicate at what stage in the process of hydration the micropyle opens. It is however necessary to draw attention to the fact that Detmer's method of arriving at this conclusion is not above criticism. He describes his experiment as follows:- "One Phaseolus seed (a), is completely immersed in water. A second seed (b) as nearly as possible equal to (a) in weight, is fixed in a suitable manner on a needle, and placed in water in such a way that the hilary apparatus is not wetted. If we weigh after a few hours, it will be found that (a) has taken up a comparatively large quantity of water, while (b) has absorbed but little." The method is subject to two very serious criticisms. In the first place the surface area in contact with water is not the same in both seeds, and secondly the method does not take

into account the possible intake of water through the hilum proper.

Mattirolo and Buscalioni (1892) working with a number of different Papilionaceous seeds concluded that water entered the seed through the hilar groove and Micropyle, while Pfäfflin (1897) considered that though water does pass through the hilar groove it is very limited in amount, and that the greater body of water which enters the seed goes through the micropyle which opens and closes according to external conditions. Eberhart (1906) who also worked on Leguminous seeds is of the opinion that the water enters the seed through the micropyle.

In the Graminaceae there also appears to be a point of easy penetration at the germinal end of the grain. For instance Schröder (1911) showed that Osmic acid and Iodine penetrated the basal end of the grain first, and suggests that the phenomenon may be explained in one of two ways. It may either be due to localized entry of these substances at the germinal end or to an increasing impermeability of the coverings from the basal to the apical end of the wheat grain. Collins (1918) carried out a number of experiments with the barley grain with a view to determining the mechanism of water intake. His experiments showed that waxing up the germinal end of the grain retarded considerably the rate of water intake, and that entry of iodine into the barley grain is not general and uniform over the whole

surface but is localized at the basal end of the grain. He concludes by saying that "the phenomena discussed justify the conclusion that the micropyle is the point of rapid entry." It is very doubtful however whether a micropyle does exist in the grain of wheat. Botanically the wheat grain is a fruit, and the external covering of the grain consists of the fruit wall in which there exists no break such as is found in the integuments of the developed ovule. It is fairly clear therefore that a micropyle as such does not exist in the grain of grasses, and that the rapid intake by the grain of barley at the germinal end must be a very permeable area in the pericarp of the grain. Brown (1931) using seeds of Lolium perenne falls into the same error when he comes to the conclusion that the earliest absorption takes place through the micropyle. According to this worker the water entering in this way diffuses upwards to a certain extent and at the higher level causes the endosperm to swell which in its turn stretches the cuticular membrane thus inducing permeability. Water entering at this higher level diffuses upwards slightly and causes permeability at a still higher level. Thus Brown considers that there is a progressive extension upwards of the area of absorption induced by the slight upward diffusion of water.

The findings of these workers are interesting in that they show that in two such widely separated

families as the Leguminosae and Gramineae, the mechanism of water intake by the seed of one has points of very close resemblance to the mechanism of water intake by the fruit of the other. Though there can be no micropyle in the latter there nevertheless appears to be an area of very rapid intake at the germinal end which is exactly the end of the leguminous seed at which the micropyle is situated. While the permeable area at the base of the grain appears to present no hindrance to the passage of Iodine and Osmic acid, to a number of other substances dissolved in water it seems to be highly selective. Under a discussion which will be embarked upon later, reference will be made to some seeds which seem either to have no micropyle or a micropyle protected below by a very efficient semipermeable layer.

In the meantine reference must be made to two hypotheses which have been put forward as a result of work on Leguminous seeds. The most recent of these is that formulated by Hamly (1932). It arose out of work on the phenomenon of "hardness in the seeds of sweet clover (Melilotus Alba). Hamly distinguished soft from hard seeds by soaking a sample in Osmic acid, which rapidly penetrates permeable areas of the coat. He found that in soft seeds the permeable center is invariably the strophiole, and that the permeability in this region is due to the

splitting apart of the elongated palisade cells. He obtained similar results with seeds of other fodder legumes. The present writer has indeed noticed that in only a very small proportion of the seed of Vicia Faba experimented with, hydration had started at the strophiole, but the great majority of seeds had other loci of commencement of hydration. It is quite apparent that the results obtained with Vicia Faba cannot be explained on the basis of Hamly's strophiole hypothesis.

In researches concerned chiefly with the semipermeability of the seed coats of Phaseolus vulgaris and Lathyrus odoratus, Atkins (1909) states with regard to the mechanism of water intake by these seeds that "the forces concerned are those of capillarity and imbibition in the initial stages, but of osmosis after germination." He considers that "seeds do not take up water by the agency of osmotic forces." Although he does not state so definitely it would appear from the graphs that he gives, that Atkin's first weighing in his weight increase experiments of hydrating seeds was taken five hours after the commencement of the soaking period, and that his second weighing was taken twelve hours after soaking commenced. In view of what we now know regarding the changes that can take place in the weight of a hydrating seed in these initial stages, reasonable doubt may be entertained as to the validity of Atkins' conclusions with regard

to the mechanism of water intake by seeds of Phaseolus and Lathyrus. The graphs Atkins gives are very similar however to those given in Chapter III, in that they show that both Bean and Pea seeds have the same phases of hydration as are found for seeds of Vicia Faba. They have an initial phase of very slow intake succeeded later by a phase of very rapid intake, periodical increases of which gradually diminish until the stage of complete hydration is reached. He does not however indicate when wrinkling commences and when dewrinkling is complete and it is therefore impossible to determine whether the changes in external appearance of the seed which accompany the changes in weight in the seed of Vicia Faba also occur in the hydrating seeds of Phaseolus and Lathyrus. But nevertheless the similarity of the graphs given by Atkins and the present writer for increase in weight of seeds during hydration would indicate that the mechanism formulated in this thesis would cover the phenomena observed by Atkins.

With reference to the permeability of the seed coverings of Phaseolus the same worker states that there is no semipermeable membrane in bean seeds till germination begins and the cell-protoplasm acts as such. The last part of this statement does not concern us here, but the first part is a direct contradiction of the findings reported in Chapter II. In this connection Shull )1913) points out that Atkins "had overlooked the open micropyles," and

Schroder (1911) in a footnote makes the same observation, but finds that the same seeds on moist sand with their micropyles turned upwards, absorbed 90% of their dry weight from 10% Sodium Chloride solution in 6 days. Atkins' method was soaking the seeds in various solutions for 43 hours and then determining any concentration which had taken place in the soaking solution by titration methods. He had no knowledge therefore of any possible changes which may have taken place in the concentration of the solution during the process of hydration. The probability is that the micropyle of the seed of Phaseobus plays the same important part in hydration as it does in Vicia Faba, and if this is in fact the case the results that Atkins obtains are understandable. The criticisms of Shull and Schroder however are only valid up to a point since it has been shown that the micropyle only opens after a certain stage in hydration is reached. The apparent support that Schroder's sand experiment gives to Atkins' findings is referable to the observation made in Chapter II, that the testa is semipermeable to begin with but as hydration proceeds the testa becomes permeable as a result of the enlargement of the intermicellar spaces. Shull (1913) used the testa of the Broad Bean and of the Scarlet Runner bean as an osmotic membrane in an osmometer, and agrees with the views here stated that the testa is not a living membrane and is not entirely semipermeable.

A number of workers have reported perfect semipermeability for the coverings of seeds of other families. In the same paper as above referred to Shull gives results of experiments with the seeds of Xanthium glabratum. He shows that a seed of this species which had been completely hydrated in water, on transference to a salt solution loses water rapidly until a balance is reached between the forces tending to draw water into the seed and those tending to draw water out of the seed. He proves also that the testa of Xanthium is a perfect semipermeable membrane. These findings are interesting in that they show that there is no micropyle in these seeds or that if there is a micropyle there can be no canal but a tissue lying behind the micropylar opening which is very highly selective in nature. Schroder (1911) finds that the covering of the wheat grain is selectively permeable while Brown (1907) and Reichard (1909) note the same for the Barley grain.

The work of Detmer (1880 and 1893) provides confirmation of the work of the present writer concerning the changes in volume of the hydrating seed. Using white giant peas in an apparatus essentially similar to that used for the experiments described in Chapter III, he says "We observe that for some time the water rises higher and higher. This goes on for three-quarters of an hour, or sometimes even for an hour and a half. Then the

water sinks for a short time, or under some conditions even for a few hours, finally again rising." The course of changes in the seed-water system as indicated in the above quotation, is identical to that observed for Vicia Faba. Detmer also agrees with the explanation given to account for the increase in volume of the seed during the wrinkling stage. His explanation is given in these terms "The testa raises itself from the cotyledons of the seed, and between the cotyledons and seed coat are formed cavities filled with rarified air so that the aggregate volume of the seeds and water must necessarily be increased." He points out that an increase in volume does not take place if peas with damaged testas are employed. With regard to the decrease in volume which takes place after a maximum has been reached he says "this must be referred to the penetration of water into cavities of the seeds", but he does not connect this penetration with the micropyle, and nor yet does he refer to the force which causes the cavities to fill up with water, Nobbe (1876) found essentially the same changes in volume taking place in his experiments with peas.

The second part of the problem with which experiments in this thesis deals is concerned with the factors which bear upon the mechanism of water intake formulated in the earlier part of this discussion. There is a definite paucity of published work on this whole part of the subject.

What work has been done, with few exceptions has not been directed specifically to the problem of the factors affecting the mechanism of water intake by seeds.

The first and probably the most important of these factors is the maturity of the seed. Statistical treatment of the figures given for the water intake of a number of seeds shows that the amount of water absorbed by each seed is approximately proportional to the air dry weight.of the seed. As against this however the amount of water absorbed per unit of air dry weight is considerably higher for small seeds than for large seeds. Jones and Bisson (1932) in the course of their researches on the development of the pea seed showed that as the developing seed approaches maturity the moisture content gradually falls until maturity is reached. A survey of the moisture content of a number of seeds of Vicia Faba on harvesting revealed the fact that the less the fresh weight of the seed the greater the proportion of water to dry matter. In fact many of the small seeds contained water to the extent of 70 or 80% of their total weights, as compared to 30-40% for large seeds. It is probable therefore that the small seed is an "immature" seed, and that the difference in quality of its colloid as compared to the large seed is responsible for its high water absorbed to dry matter ratio. Detmer (1880) says "die schwersten oder grossten Erbsen-individuen

relativ weniger Wasser bei der Quellung absorbirten, als leichtere oder kleinere." He does not however offer an explanation.

The locus of commencement of hydration of the seed has a considerable influence on the time factor in hydration. It is of importance in that the nearer the locus is to the micropyle the quicker does the micropyle open, and the sooner the latter opens after hydration commences the shorter will be the time taken for the seed to hydrate completely. Though very little is known regarding the phenomenon of localized absorption, its importance as a factor affecting the mechanism of water intake of the seed is undoubted.

A seed may remain in water for as long as a week before showing any increase in weight. Once it begins to hydrate however it will do so completely in greater or lesser time depending upon where the locus of commencement of hydration was, but in any case the time taken to complete hydration will be infinitely shorter than the time taken to the commencement of hydration. It is quite clear therefore that the latter period can have a very profound influence on the length of time the seed is actually in the water.

The period appears to be completely absent from the hydration history of a seed if the moisture content of the seed is above 20% while the less the moisture content below this figure the greater becomes the interval between commencement of soaking and hydration uhtil a critical point is reached when

further dehydration causes the period to shorten. The curtailment of the period is attributable to the formation of cracks in the seed coat as the result of stresses set up in the colloid. In this connection it is interesting to note that Hamly (1932) softens "hard" seeds of sweet clover by heating for a time below 95°C. According to this worker the treatment causes the palisade cells in the region of the strophiole to slit apart this making the seeds permeable. Again Staker (1925) recommends applying dry heat for the reduction of the percentage of hard seeds in commercial samples of lucerne seed.

Gortner (1929) describes experiments showing that the previous history of a colloid has a considerable influence on its hydration. He made up gelatine solutions of 10, 15, 20, 25, and 35% concentration, and after setting equal quantities of each in petri dishes of the same diameter, he cut out duplicate rectangles all of the same dimensions from each of the gels and allowed them to dry in a current of warm air to a moisture content which did not exceed 3.5%. He then followed the rate of water imbibition of each of the dried sections, by weighing the discs at intervals after removing surface moisture with neutral filter paper. Gortner shows that though all the discs began hydration with approximately 3% moisture, the sections which had originated from the lowest concentration imbibed least water, per grams of dry gelatine and the discs which originated

from the "solution" of highest concentration imbibed most water per gram of dried gelatine. Gortner describes other experiments showing that a disc of gelatine dried as a sol behaves quite differently in hydration from a disc of initially the same concentration which is dried as a gel to the same moisture content at a temperature below the gelating point. It is clear from these experiments that the previous treatment of a colloid system has a profound effect on their behaviour during hydration. The experiments described in Chapter IV show that although the seed is a complex of a number of colloid systems, the previous history of the seed has an important predetermining influence on its hydration. It is shown also that hysteresis of the seed colloids influences considerably the vitality of the seeds and plants produced. Recently Fox (1934) has investigated the question of storage conditions of the seed as affecting the vitality of the plant produced in greater detail than has been possible in this comprehensive survey. Using the seeds of yellow dent corn and brittle wax bean, she found that storage at high temperature produces fluctuations in the vitality of the seed. She states "These fluctuations in vitality are probably associated with the denaturing of the protoplasm," and further "denaturing and dehydration appears to take place in several steps." The interest of this work lies in the fact that similar treatment on different seeds

produces fluctuations in hydration efficiency and vitality of the seed, and whatever the explanation it is clear that the prehistory of the seed has a considerable influence on the subsequent behaviour of the seed and the plant produced by that seed.

While no work has previously been carried out on the questien of the relationship of prehistory to seed hydration, a considerable literature has grown on the relationship of seed characters and features of the plant produced. It is here tentatively suggested that a slow germinating seed produces a slow growing plant, and this conclusion is generally agreed to by most of the workers who will shortly be mentioned. But the technique adopted by these investigators, mostly of the Vienna school, is entirely different to that employed in the experiments here described. Buchinger (1927), who first described the technique, supported seeds between two glass rods in a flat dish and placed enough water or other solution in the dish so that the bottom of the glass rods were moistened. He claims that this technique is superior to the filter paper and sand-bed techniques in that it shortens the germination test. With the same apparatus he measures the "suction-force" of a seed by placing in the bottom of a series of dishes a range of sugar solutions of varying concentration. A seed which germinates in a solution of high concentration is said to have a high suction pressure and vice versa.

Using this technique he shows (1928) and (1930) that a high suction force in the seed is correlated with high crop yields while low suction force in the seed is correlated with low crop yields. Hakefost (1930) working with sugar beet and Konopa (1930) working with wheat came to the same conclusion. Pop (1930) describes experiments carried out with Buchinger's technique to show that a high suction force of seeds is associated with early maturity of the crop produced, while Sandu-Ville (1930) showed that the same was true for many Leguminosae. The same technique was used by Pammer (1928) for determining the suction force of a large number of grasses and clovers. He found that cultivated species of these plants produced seeds with higher suction force maxima than uncultivated species. Later however Pammer (1930) employed a modified Buchinger technique so as to enable him to compare the plants of the same age produced by seeds characterised by two extremes of suction-force. He shows that plants from seeds with a high suction force gave higher yields than plants produced by seeds of low suction force. Eibl (1926A) germinated seeds of various plants in sand saturated with different cane sugar solutions. He showed (1926B) that a high suction force in wheat and rye was associated with a short period of growth, and later (1927) concluded that indigenous red clover and lucerne produced seeds with a higher suction force than seeds of non-indigenous plants. The technique

described by Buchinger and employed with slight modifications by the investigators cited assumes that no change is taking place in the sugar solutions which bathe the seeds. It is submitted that the assumption is invalid, and that changes do take place in the sugar solutions as a result of the development of micro-organisms. Further the coverings of seeds are semipermeable and if the seed absorbs water from a sugar solution the osmotic concentration of the latter must become increasingly great. Chippindale (1931) recognised that micro-organisms did develop in the sugar solutions and endeavoured to keep the solution sterile by adding Sodium bicarbonate and formalin, but found that both these substances had a detrimental effect on germination.

The statement earlier made that germination is not a simple process is amply confirmed by the facts drawn attention to in this thesis. The process as a whole is affected by the history of the seed which throws its own peculiarities forward into subsequent stages of the development of the organism. The work here reported makes it quite clear that further investigation must proceed by fine analysis step by step through the process on individual seeds. The developmental history of each of the latter must be known, and this history must regard not only gross environmental factors but also such factors as the position of the seed on the plant, etc. The conception of "uniform

material" must now be regarded as a myth in so far as the significant reactions of seeds in germination are concerned. Further mere acceptance of the appearance and enumeration of plantlets as a criterion of germination success is definitely valueless.

## Chapter VII.

## Bibliography.

Atkins W.R.G. (1909) "The absorption of water by seeds". Scient. Proc. Royal Dublin Soc. 12.35.46. 1909.

Banister H. (1929) "Elementary applications of Statistical Method." Blackie & Son, Ltd., London 1929.

Beck G. (1878) "Vergleichende Anatomie der Samen von Vicia und Ervum." Sitzb. der Mathm. - Naturwiss-ensch. Classe der Kaiserlischen Akad. der Wissensch. Wien. 77.545-579.1878

Bischoff G.W. (1844) "Handbuch der Bot. Terminologie und Systemkunde." 3 vols. Nurnberg 1833-44.

Brown A.J. (1907) "On the existence of a semi-permeable membrane enclosing the seeds of some Gramineae." Anns. Bot. 21. 80-87. 1907.

Brown R. (1931) "The absorption of water by seeds of Lolium perenne and certain other Gramineae." Anna. Appl. Biol. 18.559-573. 1931.

Buchinger A. (1927) "Eine neuer Keimapparat." Fort. der Land. 2.192-194.1927 (Original not seen. Abstract in Bot. Central Bl. 10.319. 1927).

(1928) "Selection nach der Saugkraft" Fort. der. Lond. 3.1065-67. 1928. (Original not seen. Abstract in Bot Centralbl. 14.265.1929).

(1930) "Die Bedeutung der Selection nach der Saugkraft fur die Pflanzenzuchtung." Zeit. fur Pflanzenzuchtung. 5.101.1930.

Chippindale H.G.(1931) "Suction - force measurements on the seeds of some strains of grasses." Welsh Journ. Agric. 7.168-182. 1931.

Collins E.J. (1918) "The structure of the integ-umentary system of the Barley grain in relation to localized water absorption and semi-permeability." Anns. Bot. 32.381-414. 1918.

Detmer W. (1880) "Vergleichende Physiologie des Keimungsprocesses der Samen." Gustav Fischer, Jena.. 1880.

(1898) "Practical Plant Physiology" Translated from second German edition by S.A. Moor. Swan Sonnenschein & Co. Ltd., London. 1898.

Eberhart C. (1906) "Untersuchungen uber das Varquellen der Samen." Tuang. Diss. Jena. 1906. (Original not seen. Abstracts in Bot. Jahresbericht. 34 (2).525. 1906 and Bot. Centralbl. 115.369 -370. 1907).

Eibl. A. (1926A) "Osmotische und Saugkraftmessungen an Kulturpflanzen I". Fort. der. Land. 1.269-272.1926. (Original not seen. Abstract in Bot Centralbl. 8.172. 1926).

(1926B) "Osmotische und Saugkraftmessungen an Kulturpflanzen II. Getreide." Fort. der Land. 1.661-669. 1926. (Original not seen. Abstract in Bot. Centralbl. N. Folge. 9.303. 1927).

(1927) "Osmotische und Saugkraftmessungen an Kulturpflanzen. IV Rotklee und Luzerne." Fort. der Land. 2.123-124. 1927. (Original not seen. Abstract in Bot. Centralbl. 10.206.1927).

Fisher R.A. (1932) "Statistical Methods for Research Workers." 4th Edition. Oliver & Boyd, Edinburgh 1932.

Fox, L.E. (1932) "The effects of high temperatures on seeds." Amer. Journ. Bot. 21.711. 1934.

Gortner R.A. (1929) "Outlines of Biochemistry." John Wiley & Sons, New York 1929.

Hakefost G. (1930) "Der Zusammenhang von Saugkraft und Leistungsfahigkeit dargestellt an zwanzig Zuckerrubenstammen." Fort. der. Land. 5.680. 1930. (Original not seen Abstract in Bot. Centralbl. N. Folge. 18.313. 1931).

Hamly D.H. (1932) "Softening of the seeds of Melilotus Alba." Bot. Gaz. 93.345-375. 1932.

Harz C.D. (1885) "Landwirthschaftliche Samenkunde." 2. Vols. Berlin 1885.

Jones H.A. and Bisson C.S. (1932) "Changes accompanying fruit development in the garden Pea" Plant Phys. 7.91-105. 1932.

Konopa H. (1930) "Sangkraftmessungen an einigen Weizen und Roggensorten." Fortschr. der. Landwirth. 5.707 1930. (Original not seen. Abstract in Bot. Centralbl. N. Folge 18. 326. 1931).

Mattirolo O and Buscalioni L. (1892) "Richerche anatomo - fisiologische sui tegumenti seminali delle Papilionaceae" Reale Accad. delle Sci. di Torino II. 42. 223-318 and 359-455. 1892.

Nelson A (1926) "Hard seeds in Leguminosae" Nature 118.804-805. 1926.

Nelson A and Macsween J.C. (1933) "Hard seeds and broken seedlings in Red Clover. (Trifolium Rratense). IV Early stages of Germination (Physical)." Procs. Bot. Soc. Edin. 31.247-255. 1933.

Nierenstein M (1927) "Allen's Commercial Organic Analysis" Vol V. J. & A. Churchill, London 1927.

Nobbe F. (1876) "Handbuch der Samenkunde." Berlin 1876.

Overhage C. (1887). "Anatomischer Untersuchungen und Keimungsgeschichte der Samen von Canna und Musa." Inaug. Diss. Erlangen. (1887) (Original not seen. Abstract in Bot. Jahresb. 16.744.1888)

Pammel L.HL (1899) "Anatomical characters of the seeds of Leguminosae, chiefly genera of Gray's Manual." Trans. Acad. Sci. St. Louis. 9.91-274.1899.

Pammer F. (1928) "Osmotiche und Saugkraftmessungen. VII Grasser und Leguminosen." Fort. der Laudwirthsch. 3.441-448. 1928. (Original not seen. Abstract in Bot. Centralbl. N Folge. 13.199. 1928).

(1930) "Der Osmotische Wert als Selektionsmoment bei Futterpflanzen." Zeitschr. fur. Pflanzenzuchtung 5.115.1930.

Pfäfflin P. (1897) "Untersuchungen uber Entwicklungsgeschichte, Bau und Function der Nabelspalte und darunter liegenden Tracheideninsel, verschiedener praktisch wichtiger Papilionaceen Samen." Inaug. Diss. Bern. 1897. (Original not seen. Abstracf in Bot. Jahresbericht. 25. 529 -530 1897, and in Beih z. Bot. Centralbl 7.344-5 1898).

Plimmer R.H.A (1918) "Practical Organic and Biochemistry." Longmans Green & Co., London 1918.

Pop G. (1930) "Saugkraft der Weizen" Fortschr der landwirth. 5.125-129.1930. (Original not seen. Abstract in Bot. Centralbl. N. Folge 17.75 1930).

Pringsheim E.G.(1930) "Untersuchungen uber Samenquellung. I Die Abhangigheit der quellung von der Beschaffenheit der Samen und vom Medium." Planta (Abt, E.Z. wissen.Biol) 11.528-581.1930.

Pringsheim N. (1848) "De forma et incremento stratorum crassiorum in plantarum cellula, observationes quaedam novae." Linnaea 21.145-180. 1848.

Reichard A. (1909) "Hat der Gerbstoff der Samenhaut des Gestenkorns eine Anteil an der Halbdurchlassigkeit dieser Membran?" Zeitschr. Gesamte Brauw. 33.145-148, 157-160. 1909.

Sandu-Ville C (1930) "Saugkraftmessungen an Leguminosen." Fortschr. der Land. 5.129-131. 1930. (Original not seen. Abstract in Bot. Centralbl N. Folge 17.75.1930)

Schroder H. (1911) "Uber der Selektiv permeable Hulle des Weizenkornes" Flora 2.186-208. 1911.

Sempolowski A. (1874) "Beitrage zur Kenntniss des Blaues der Samenschale" Inaug. Diss. Leipzig. 1874.

Shull C.A. (1913) "Semipermeability of Seed Coats." Bot. Goz. 56.169-199.1913.

Staker E.V. (1925) "The effect of dry heat on alfalfa seed and its adulterants." Journ. Amer. Soc. Agron. 17.23-40.1925.

Tippett L.H.C. (1931) "The Methods of Statistics" Williams and Norgate Ltd., London 1931.

------oOo------